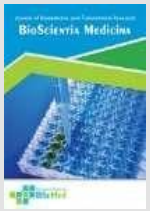
eISSN (Online): 2598-0580



Bioscientia Medicina: Journal of Biomedicine & Translational Research

Journal Homepage: www.bioscmed.com

Ki-67 and HER2-Negative Status as Predictive Factors for Recurrence and Progression in Breast Cancer: Implications for Treatment Strategies

Chemy Wiryawan Cahyono[1*], Selamat Budijitno[2]

[1]Surgery Specialized Residency Education Program, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Semarang, Indonesia

[2]Department of Surgery, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Semarang, Indonesia

ARTICLE INFO

Keywords:
Breast cancer
HER2-negative
Ki-67
Progression
Recurrence

***Corresponding author:**
Chemy Wiryawan Cahyono

E-mail address:
chemyhelvi@gmail.com

All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/bsm.v9i5.1266

A B S T R A C T

Background: Breast cancer remains a significant health challenge, with HER2-negative subtypes representing a majority of cases. Ki-67, a marker of cell proliferation, has emerged as a potential prognostic factor in various cancers, including breast cancer. This study aimed to evaluate the association of Ki-67 expression and HER2-negative status with tumor characteristics, treatment response, and disease progression in breast cancer patients. **Methods:** A retrospective cross-sectional study was conducted at Dr. Kariadi General Hospital, Semarang, Indonesia. Data from 94 patients diagnosed with breast cancer were collected, including immunohistochemical profiles, HER2 status, Ki-67 index, treatment regimens, and tumor size. Statistical analysis was performed using SPSS version 25.0 for Windows. **Results:** The majority of patients (50%) presented with Luminal B-type breast cancer, and 77.7% had a Ki-67 index >20%. HER2-negative status was observed in 78.7% of patients. No significant correlation was found between HER2-negative status and the type of therapy given (p=0.131) or tumor size (p=0.467). Similarly, Ki-67 expression >20% did not correlate significantly with the type of therapy (p=0.070) or tumor size (p=0.156). **Conclusion:** While Ki-67 and HER2-negative status are recognized as important prognostic factors in breast cancer, this study did not find a significant association with treatment modalities or tumor size in the studied population. Further research with a larger sample size and longer follow-up is needed to validate these findings and explore the complex interplay of Ki-67, HER2 status, and other clinical variables in breast cancer progression.

1. Introduction

Breast cancer, a complex and heterogeneous disease characterized by uncontrolled cell growth and proliferation, remains a significant global health challenge. It is the most frequent malignancy among women worldwide, with varying incidence and mortality rates across different regions. The human epidermal growth factor receptor 2 (HER2) plays a crucial role in breast cancer development, and its overexpression is associated with aggressive tumor behavior and poorer prognosis. HER2-negative breast cancers, however, represent the majority of cases, and their biological and clinical characteristics differ from HER2-positive tumors.[1-5]

Ki-67, a nuclear protein expressed in proliferating cells, has emerged as a potential prognostic and predictive marker in various cancers, including breast cancer. Its expression level reflects the proliferative activity of tumor cells and has been linked to tumor grade, stage, and response to therapy. Several studies have investigated the role of Ki-67 in breast cancer, with some suggesting its association with disease-free survival, overall survival, and response to neoadjuvant chemotherapy. However, the prognostic and predictive

value of Ki-67 in HER2-negative breast cancer remains controversial, and further research is needed to clarify its clinical significance.[6-10] This study aimed to evaluate the association of Ki-67 expression and HER2-negative status with tumor characteristics, treatment response, and disease progression in breast cancer patients at Dr. Kariadi General Hospital, Semarang, Indonesia.

2. Methods

This study employed a retrospective cross-sectional design, utilizing data collected from patient medical records and pathology reports. The study was conducted at Dr. Kariadi General Hospital, a tertiary care hospital located in Semarang, Indonesia. The study population consisted of female patients diagnosed with breast cancer at Dr. Kariadi General Hospital between January 2020 and December 2023. To be eligible for inclusion in the study, patients had to meet the following criteria; Histologically confirmed diagnosis of invasive breast cancer; Age 18 years or older at the time of diagnosis; Availability of complete medical records, including pathology reports, treatment information, and follow-up data; Patients with a history of other malignancies or those who received neoadjuvant chemotherapy were excluded from the study.

A total of 94 patients met the eligibility criteria and were included in the final analysis. All eligible patients were included in the study, and no specific sampling technique was employed. Data were collected from patient medical records and pathology reports using a standardized data collection form. The following variables were extracted; Demographic information: Age at diagnosis, gender, ethnicity, and menopausal status; Tumor characteristics: Tumor size, histological grade, lymph node status, and immunohistochemical (IHC) profile (estrogen receptor (ER), progesterone receptor (PR), and HER2 status); Ki-67 index: Percentage of tumor cells positive for Ki-67 staining, assessed by IHC; Treatment information: Type of surgery (breast-conserving surgery or mastectomy), chemotherapy regimen (if applicable), radiotherapy, and hormonal therapy; Disease progression: Local recurrence, distant metastasis, and disease-free survival.

Immunohistochemical (IHC) staining was performed on formalin-fixed, paraffin-embedded tumor tissue sections to assess the expression of ER, PR, HER2, and Ki-67. The staining procedures were conducted according to the manufacturers' instructions. The scoring of ER and PR expression was based on the Allred scoring system, which combines the proportion and intensity of staining. HER2 status was evaluated according to the American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) guidelines. Ki-67 index was determined by counting the percentage of positively stained tumor cells in at least 1,000 cells.

Data were analyzed using SPSS version 25.0 for Windows. Descriptive statistics were used to summarize patient characteristics and tumor features. The Chi-square test was used to assess the association between HER2-negative status, Ki-67 expression, type of therapy, and tumor size. A p-value <0.05 was considered statistically significant. This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Institutional Review Board of Dr. Kariadi General Hospital. Patient confidentiality was maintained throughout the study, and all data were anonymized.

3. Results

Table 1 provides a breakdown of the characteristics of the 94 breast cancer patients included in the study at Dr. Kariadi General Hospital Semarang; Immunohistochemical Profile: Luminal B is the most common subtype, representing 50% of the cases. This subtype is known to be more aggressive than Luminal A. Triple-negative breast cancer accounts for 23.4% of the cases. This subtype lacks expression of estrogen receptors, progesterone receptors, and HER2, making it more challenging to treat with targeted therapies. Luminal A is the least prevalent subtype in this cohort, comprising 14.9% of the cases. HER2-Enriched

tumors make up 11.7% of the cases; HER2-Type: The majority of patients (78.7%) are HER2-Negative. This is important as HER2-targeted therapies would not be effective for these individuals. Only 19.1% of patients are HER2-Positive, indicating that they could potentially benefit from HER2-targeted treatments. A very small percentage (2.1%) shows HER2 Over-Expression; Ki-67 index: Most patients (77.7%) have a Ki-67 index >20%. This suggests a high proliferation rate among the tumor cells, which is generally associated with a less favorable prognosis; Type of therapy given: More than half of the patients (56.4%) have received First-line chemotherapy. 28.7% are Not yet given chemotherapy, which might include patients undergoing initial surgery or those with less aggressive disease. 14.9% have received Second-line chemotherapy, indicating that they have experienced disease progression or recurrence after initial treatment; Size of breast cancer: The average tumor size is 1017.20 ± 1354.59 cm^2. However, the large standard deviation suggests a wide range of tumor sizes in the sample.

Table 1. Characteristics of breast cancer patients at Dr. Kariadi General Hospital Semarang.

Variable	n (%)	Mean ± SD
Immunohistochemical profile		
Triple negative	22 (23.4%)	
Luminal A	14 (14.9%)	
Luminal B	47 (50.0%)	
HER2-enriched	11 (11.7%)	
HER2-type		
Positive	18 (19.1%)	
Negative	74 (78.7%)	
Over-expressed	2 (2.1%)	
Ki-67 index		
<20%	21 (22.3%)	
>20%	73 (77.7%)	
Type of therapy given		
Not yet given chemotherapy	27 (28.7%)	
First-line chemotherapy	53 (56.4%)	
Second-line chemotherapy	14 (14.9%)	
Size of breast cancer		1017.20 ± 1354.59 cm^2

Table 2 provides a more detailed view of the breast cancer patients at Dr. Kariadi General Hospital by examining the relationship between HER2 status and several key variables: immunohistochemical profile, Ki-67 index, type of therapy, and tumor size; Immunohistochemical Profile: As expected, all HER2-Enriched tumors are HER2-Positive. Interestingly, half of the Luminal B tumors also fall into this category, suggesting a more aggressive subtype within the Luminal B group. Triple-negative and Luminal A tumors are exclusively HER2-Negative. The majority of Luminal B tumors (51.4%) are also HER2-Negative. The HER2-Overexpressed category has a very small sample size, with only two cases observed, both belonging to the HER2-Enriched subtype; Ki-67 Index: A higher proportion of HER2-Negative tumors (25.7%) have a Ki-67 index <20% compared to HER2-positive tumors (11.1%). This suggests that HER2-positive tumors tend to have a higher proliferation rate. The majority of both HER2-Positive (88.9%) and HER2-Negative (74.3%) tumors have a Ki-67 index >20%, indicating a high proliferation rate in most cases; Type of Therapy Given: The distribution of patients across different therapy stages (not yet given, first-line, second-line) is quite similar between HER2-Positive and HER2-Negative groups. This suggests that HER2

status alone might not be the sole determinant of treatment decisions in this cohort; Size of Breast Cancer (cm^2): HER2-Over Expressed tumors appear to be larger on average, but the small sample size makes it difficult to draw firm conclusions. The average size of HER2-Negative tumors is slightly larger than HER2-Positive tumors, although the large standard deviations in both groups indicate significant variability.

Table 2. Overview of immunohistochemical profile, HER2-type, Ki-67 index, type of therapy, and size of breast cancer.

Variable	HER2-Positive	HER2-Negative	HER2-Over Expressed
Immunohistochemical profile			
Triple-negative	0	22 (29.7%)	
Luminal A	0	14 (18.9%)	
Luminal B	9 (50.0%)	38 (51.4%)	
HER2-enriched	9 (50.0%)	0	2 (100%)
Ki-67 index			
<20%	2 (11.1%)	19 (25.7%)	
>20%	16 (88.9%)	55 (74.3%)	2 (100%)
Type of therapy given			
Not yet given chemotherapy	3 (16.7%)	23 (31.1%)	1 (50.0%)
First-line chemotherapy	53 (56.4%)	43 (58.1%)	1 (50.0%)
Second-line chemotherapy	14 (14.9%)	8 (10.8%)	0
Size of breast cancer (cm^2)	754.11 ± 1223.30	1069.96 ± 1400.77	1433.00 ± 363.45

Table 3 focuses on the association of HER2-negative status and Ki-67 expression with two key factors: the type of therapy given and tumor size; Therapy: The p-value of 0.131 indicates that there is no statistically significant association between HER2-negative status and the type of therapy given. This means that HER2-negative status does not appear to influence whether a patient receives no chemotherapy, first-line chemotherapy, or second-line chemotherapy. Similarly, the p-value of 0.070 suggests that there is no statistically significant association between Ki-67 expression above 20% and the type of therapy received; Tumor Size (cm^2): Again, the p-value of 0.467 demonstrates no statistically significant association between HER2-negative status and tumor size. This implies that HER2-negative tumors are not more likely to be of a particular size category (<500, 500-1500, >1500 cm^2). The p-value of 0.156 indicates no statistically significant association between Ki-67 expression above 20% and tumor size.

Table 3. Association of HER2-negative status and Ki-67 expression with treatment and tumor size.

Variable	HER2-Negative	Ki-67 >20%
Therapy		
No chemotherapy	18 (24.3%)	10 (13.7%)
First-line chemotherapy	40 (54.1%)	45 (61.6%)
Second-line chemotherapy	6 (8.1%)	8 (11.0%)
p-value	131	70
Tumor size (cm^2)		
< 500	15 (20.3%)	12 (16.4%)
500 - 1500	35 (47.3%)	38 (52.1%)
> 1500	14 (18.9%)	13 (17.8%)
p-value	467	156

4. Discussion

In this study, the distribution of breast cancer subtypes revealed a predominance of Luminal B tumors, followed by Triple-Negative, Luminal A, and HER2-enriched subtypes. This pattern aligns with observations from previous research conducted in Indonesia and other Southeast Asian countries, where Luminal B has been identified as the most prevalent subtype. Luminal B tumors, characterized by the presence of estrogen receptors (ER+) and/or progesterone receptors (PR+), are driven by hormonal influence. They also exhibit a higher expression of Ki-67, a marker of cellular proliferation, compared to other subtypes. This elevated Ki-67 expression is associated with a more aggressive clinical course and a poorer prognosis compared to Luminal A tumors, which also express hormone receptors but have lower Ki-67 levels. The significant proportion of Luminal B tumors in this study population underscores the urgent need for effective treatment strategies specifically tailored to this subtype. To elaborate further, Luminal B breast cancer is a subtype characterized by the presence of estrogen receptors (ER) and/or progesterone receptors (PR) on the tumor cells, indicating that these cancers are hormone-sensitive. This subtype also exhibits a higher expression of Ki-67, a marker of cellular proliferation, compared to Luminal A tumors. The higher Ki-67 expression in Luminal B tumors is associated with a more aggressive clinical course, a higher risk of recurrence, and a poorer prognosis compared to Luminal A tumors. The prevalence of Luminal B breast cancer varies across different populations and geographic regions. In Western countries, Luminal A is the most common subtype, while Luminal B is less prevalent. However, in Asian populations, including Indonesia, Luminal B has been reported to be the most frequent subtype. The reasons for this variation are not fully understood but may be related to differences in genetic predisposition, lifestyle factors, and environmental exposures. The significant proportion of Luminal B tumors in this study population highlights the importance of developing effective treatment strategies specifically tailored to this subtype. Hormone therapy, such as tamoxifen or aromatase inhibitors, is the mainstay of treatment for Luminal B breast cancer. However, many patients with Luminal B tumors experience disease recurrence or progression despite hormone therapy. Therefore, there is a need for additional therapeutic approaches to improve outcomes for patients with this subtype. Several ongoing research efforts are focused on identifying new therapeutic targets and developing novel treatment strategies for Luminal B breast cancer. These include targeted therapies that inhibit specific signaling pathways involved in tumor growth and progression, as well as immunotherapies that harness the body's immune system to fight cancer. Constituting a significant portion of cases, Triple-negative breast cancer (TNBC) is defined by the absence of estrogen receptors (ER-), progesterone receptors (PR-), and human epidermal growth factor receptor 2 (HER2-). This subtype presents a unique therapeutic challenge due to the lack of targets for hormone therapy and HER2-targeted therapy, which are effective in other subtypes. TNBC tends to be more aggressive and is associated with a higher risk of early recurrence and distant metastasis compared to other subtypes. The relatively high prevalence of TNBC in this study population emphasizes the need for alternative treatment approaches and ongoing research to improve outcomes for patients with this aggressive form of breast cancer. TNBC is a heterogeneous disease with diverse molecular and genetic characteristics. Several subtypes of TNBC have been identified based on gene expression profiling, each with distinct clinical features and potential therapeutic vulnerabilities. Ongoing research is focused on understanding the molecular mechanisms underlying TNBC development and progression, with the goal of identifying new therapeutic targets and developing more effective treatment strategies. Current treatment options for TNBC include chemotherapy, radiation therapy, and surgery. However, the prognosis for patients with TNBC remains poor compared to other subtypes,

highlighting the urgent need for novel therapeutic approaches. Immunotherapy, which harnesses the body's immune system to fight cancer, has shown promising results in some patients with TNBC and is an area of active investigation. Representing the least prevalent subtype in this cohort, Luminal A tumors share similarities with Luminal B tumors in their expression of hormone receptors (ER+ and/or PR+) but are distinguished by their lower Ki-67 expression. This lower proliferation rate generally translates to a less aggressive clinical course and a better prognosis compared to Luminal B tumors. While hormone therapy remains the mainstay of treatment for Luminal A breast cancer, ongoing research continues to explore the optimal duration and choice of endocrine therapy. Luminal A breast cancer is the most common subtype of breast cancer, accounting for approximately 60-70% of all cases. It is characterized by the presence of estrogen receptors (ER) and/or progesterone receptors (PR) on the tumor cells, indicating that these cancers are hormone-sensitive. Luminal A tumors typically have a lower expression of Ki-67, a marker of cellular proliferation, compared to Luminal B tumors. The lower Ki-67 expression in Luminal A tumors is associated with a less aggressive clinical course, a lower risk of recurrence, and a better prognosis compared to Luminal B tumors. Hormone therapy, such as tamoxifen or aromatase inhibitors, is the mainstay of treatment for Luminal A breast cancer. The choice of hormone therapy depends on several factors, including the patient's menopausal status, the risk of recurrence, and the presence of any contraindications. The optimal duration of hormone therapy for Luminal A breast cancer is still under investigation. Current guidelines recommend 5-10 years of adjuvant hormone therapy for most patients with Luminal A tumors. However, some studies suggest that extending hormone therapy beyond 10 years may further reduce the risk of recurrence, particularly in patients with high-risk features. HER2-enriched tumors are characterized by the overexpression of HER2, a receptor tyrosine kinase that plays a crucial role in regulating cell growth and division. HER2 overexpression is associated with aggressive tumor behavior, increased risk of recurrence, and a poorer prognosis. However, the advent of HER2-targeted therapies, such as trastuzumab, has dramatically improved outcomes for patients with HER2-positive breast cancer. The identification of HER2-enriched tumors in this study population highlights the importance of HER2 testing and the potential benefit of HER2-targeted therapies for these patients. HER2-positive breast cancer accounts for approximately 20-25% of all breast cancer cases. It is characterized by the overexpression of HER2, a receptor tyrosine kinase that promotes cell growth and division. HER2 overexpression is associated with aggressive tumor behavior, increased risk of recurrence, and a poorer prognosis compared to HER2-negative breast cancer. The development of HER2-targeted therapies, such as trastuzumab, has revolutionized the treatment of HER2-positive breast cancer. These therapies work by blocking the activity of HER2, thereby inhibiting tumor growth and progression. The use of HER2-targeted therapies has significantly improved outcomes for patients with HER2-positive breast cancer, leading to increased survival rates and improved quality of life. Observed in a majority of patients (78.7%), HER2-negative status aligns with global trends where most breast cancers lack HER2 overexpression. HER2-negative breast cancers represent a heterogeneous group with diverse biological and clinical characteristics. Compared to HER2-positive tumors, they generally have a lower risk of recurrence and a better prognosis. However, HER2-negative tumors are also less responsive to HER2-targeted therapies, which have revolutionized the treatment of HER2-positive breast cancer. This underscores the importance of understanding the role of other prognostic and predictive factors, such as Ki-67, in HER2-negative breast cancer to optimize treatment strategies and improve outcomes for this patient population. HER2-negative breast cancer is a heterogeneous group of tumors that do not overexpress the HER2 protein. This group includes

various subtypes, such as Luminal A, Luminal B, and Triple-Negative breast cancer. The prognosis for patients with HER2-negative breast cancer is generally better than for those with HER2-positive tumors, but it still varies depending on the specific subtype and other factors such as tumor size, grade, and lymph node involvement. Treatment strategies for HER2-negative breast cancer depend on the specific subtype and risk factors. Hormone therapy is the mainstay of treatment for hormone receptor-positive (ER+ and/or PR+) tumors, while chemotherapy may be considered for patients with high-risk features or triple-negative tumors. The majority of patients (77.7%) exhibited a Ki-67 index >20%, indicating a high proliferation rate among the tumor cells. Ki-67, a nuclear protein expressed in all phases of the cell cycle except for the resting phase (G0), serves as a marker of cellular proliferation. Its expression level reflects the proliferative activity of tumor cells, providing valuable information about tumor growth and aggressiveness. Higher Ki-67 expression has been consistently associated with more aggressive tumor behavior, increased risk of recurrence, and poorer prognosis in various cancers, including breast cancer. The high prevalence of Ki-67 expression >20% in this study population suggests a need for close monitoring and potentially more aggressive treatment approaches for these patients. Ki-67 is a nuclear protein that is expressed in all phases of the cell cycle except for the resting phase (G0). It is therefore a marker of cellular proliferation, and its expression level reflects the proportion of cells that are actively dividing. In breast cancer, Ki-67 expression has been shown to be an independent prognostic factor, with higher expression associated with a higher risk of recurrence and poorer survival outcomes. The prognostic value of Ki-67 has been demonstrated in various subtypes of breast cancer, including Luminal A, Luminal B, and Triple-Negative breast cancer. In Luminal A tumors, Ki-67 expression is typically low, and these tumors generally have a good prognosis. In Luminal B tumors, Ki-67 expression is higher, and these tumors are more likely to recur and metastasize. In Triple-Negative breast cancer, Ki-67 expression is often high, and these tumors are associated with a poor prognosis. The predictive value of Ki-67 is still under investigation, but some studies suggest that it may be useful in identifying patients who are more likely to benefit from chemotherapy. For example, in patients with Luminal B tumors, a high Ki-67 expression may indicate a greater benefit from chemotherapy in addition to hormone therapy. The distribution of tumor subtypes, HER2 status, and Ki-67 expression in this study population provides valuable insights into the characteristics of breast cancer patients at Dr. Kariadi General Hospital. The high prevalence of Luminal B and Triple-Negative subtypes, coupled with the high proportion of patients with Ki-67 expression >20%, highlights the need for tailored treatment strategies and ongoing research to improve outcomes for these patients. The findings also underscore the importance of comprehensive tumor profiling, including immunohistochemical analysis and Ki-67 assessment, to guide treatment decisions and personalize care for breast cancer patients. By understanding the specific characteristics of each tumor subtype and the prognostic and predictive significance of markers such as HER2 and Ki-67, clinicians can make more informed treatment decisions. This personalized approach to breast cancer management aims to optimize treatment efficacy and minimize unnecessary side effects by tailoring therapies to the individual needs of each patient.[11-14]

In this study, we sought to explore the relationship between HER2-negative status, Ki-67 expression, and clinical parameters such as treatment modalities and tumor size. Contrary to our initial expectations, we did not observe a statistically significant association between HER2-negative status and the type of therapy given (p=0.131) or tumor size (p=0.467). Similarly, Ki-67 expression levels above 20% did not show a significant correlation with the type of therapy (p=0.070) or tumor size (p=0.156). This finding diverges from some previous studies that have reported a correlation between Ki-67 expression and

response to neoadjuvant chemotherapy, particularly in HER2-negative patients. However, it is important to acknowledge that the literature on this topic presents conflicting results, and the prognostic and predictive value of Ki-67 in HER2-negative breast cancer remains a subject of ongoing debate. Several factors may contribute to the lack of significant associations observed in our study. First, the sample size was relatively small, which may have limited the statistical power to detect subtle associations. Second, the retrospective nature of the study introduces limitations, as treatment decisions are often influenced by a multitude of factors not captured in the medical records, such as patient preferences, comorbidities, and physician experience. Third, the study population was heterogeneous, encompassing patients with different tumor subtypes, stages, and treatment regimens, which may have masked potential associations between Ki-67, HER2 status, and treatment outcomes. Despite the absence of statistically significant associations, it is crucial to emphasize that HER2-negative status and Ki-67 expression remain important prognostic factors in breast cancer. HER2-negative tumors generally exhibit a more favorable prognosis compared to HER2-positive tumors. Moreover, Ki-67 expression has been shown to be an independent predictor of disease-free survival and overall survival in some studies. Consequently, these factors should continue to be considered in clinical decision-making processes, even if they do not directly dictate treatment choices. Treatment decisions in breast cancer are complex and multifaceted, involving careful consideration of various factors beyond HER2 status and Ki-67 expression. The presence or absence of estrogen receptors (ER) and progesterone receptors (PR) plays a crucial role in determining the suitability of hormone therapy, such as tamoxifen or aromatase inhibitors. Hormone therapy is highly effective in hormone receptor-positive tumors, but its efficacy is limited in hormone receptor-negative tumors. The extent of tumor spread (stage) and the degree of cellular differentiation (grade) provide important prognostic information and guide treatment decisions. Early-stage tumors may be amenable to breast-conserving surgery, while advanced-stage tumors may require mastectomy and systemic therapy. High-grade tumors are generally more aggressive and may require more intensive treatment. The presence or absence of cancer cells in the lymph nodes is a critical prognostic factor and influences treatment decisions. Lymph node involvement may necessitate additional surgery, radiation therapy, or chemotherapy. Patient age, overall health status, comorbidities, and personal preferences also play a significant role in treatment decisions. Older patients or those with significant comorbidities may not be suitable candidates for aggressive treatments. Treatment decisions are also influenced by the experience and expertise of the treating physician, as well as institutional guidelines and protocols. Ki-67 has emerged as a potential prognostic and predictive marker in breast cancer, but its precise role in clinical decision-making remains a subject of ongoing debate. Some studies have suggested that Ki-67 expression may be useful in predicting response to neoadjuvant chemotherapy, particularly in HER2-negative patients. However, other studies have failed to demonstrate a clear association between Ki-67 and treatment response. The variability in findings across studies may be attributed to several factors, including differences in study design, patient populations, treatment protocols, and Ki-67 assessment methods. There is also a lack of standardization in Ki-67 cutoff values, with different studies using different thresholds to define high and low expressions. Despite the ongoing debate, Ki-67 remains a valuable prognostic marker in breast cancer. Higher Ki-67 expression is generally associated with a higher risk of recurrence and poorer survival outcomes, regardless of tumor subtype. Therefore, Ki-67 should be considered alongside other prognostic factors when making treatment decisions. To further elaborate on the complexities surrounding Ki-67, it's important to understand its biological role and the challenges associated with its measurement and interpretation. Ki-67 is a nuclear protein that is

expressed in all actively proliferating cells. It is involved in various cellular processes, including cell cycle progression, DNA replication, and chromosome segregation. In cancer cells, Ki-67 expression is often upregulated, reflecting the increased proliferative activity of these cells. The level of Ki-67 expression can vary significantly between different tumor types and even within the same tumor. This variability can be attributed to several factors, including the underlying genetic and molecular characteristics of the tumor, the tumor microenvironment, and the presence of other factors that influence cell proliferation. Different laboratories may use different staining protocols and antibodies for Ki-67 immunohistochemistry, which can lead to variability in staining results. The scoring of Ki-67 expression is often subjective, as it relies on visual assessment of the percentage of positively stained cells. This can lead to interobserver variability in scoring, particularly when the staining is heterogeneous. There is no universally accepted cutoff value for Ki-67 expression. Different studies have used different thresholds to define high and low expression, which can make it difficult to compare results across studies. Ki-67 expression can change over time, particularly in response to treatment. This can make it challenging to interpret Ki-67 expression levels in the context of treatment decision-making. HER2-negative breast cancer is a heterogeneous group of tumors that do not overexpress the HER2 protein. This group includes various subtypes, such as Luminal A, Luminal B, and Triple-Negative breast cancer. The prognosis for patients with HER2-negative breast cancer is generally better than for those with HER2-positive tumors, but it still varies depending on the specific subtype and other factors such as tumor size, grade, and lymph node involvement. Treatment strategies for HER2-negative breast cancer depend on the specific subtype and risk factors. Hormone therapy is the mainstay of treatment for hormone receptor-positive (ER+ and/or PR+) tumors, while chemotherapy may be considered for patients with high-risk features or triple-negative tumors. The heterogeneity of HER2-negative breast cancer underscores the need for personalized treatment approaches. Factors such as hormone receptor status, tumor grade, Ki-67 expression, and patient preferences should be considered when making treatment decisions.[15-17]

The findings of this study have implications for treatment strategies in breast cancer, particularly for patients with HER2-negative tumors. While Ki-67 and HER2-negative status may not be independent predictors of treatment response or tumor size in all patient populations, they remain important prognostic factors that should be considered in clinical decision-making. Further research is needed to identify specific subgroups of patients who may benefit from targeted therapies based on their Ki-67 and HER2 status. For patients with HER2-negative breast cancer, treatment decisions should be individualized based on a comprehensive assessment of tumor characteristics, patient factors, and available treatment options. Hormone receptor-positive (ER+ and/or PR+) tumors are generally treated with endocrine therapy, such as tamoxifen or aromatase inhibitors, to block the effects of estrogen on tumor growth. Chemotherapy may also be considered for patients with high-risk features, such as large tumor size, high grade, or lymph node involvement. Hormone receptor-positive tumors are eligible for endocrine therapy, which has been shown to significantly improve outcomes in this patient population. The choice of endocrine therapy depends on several factors, including the patient's menopausal status, the risk of recurrence, and the presence of any contraindications. Tumor size, grade, and lymph node involvement are important prognostic factors that can guide treatment decisions. Larger tumors, higher-grade tumors, and lymph node involvement may indicate a need for more aggressive treatment, such as chemotherapy. Patient age, overall health status, comorbidities, and personal preferences should also be considered when making treatment decisions. Older patients or those with significant comorbidities may not be suitable candidates for aggressive treatments. Although the role of Ki-67 in treatment decision-making for HER2-negative breast cancer is

still evolving, some studies suggest that it may be useful in identifying patients who are more likely to benefit from chemotherapy. However, more research is needed to validate these findings and establish clear guidelines for using Ki-67 to guide treatment decisions. The role of Ki-67 in treatment decision-making for HER2-negative breast cancer is still under investigation. Some studies suggest that Ki-67 may help identify patients who are more likely to benefit from chemotherapy, particularly in the neoadjuvant setting. However, more research is needed to validate these findings and establish clear guidelines for using Ki-67 to guide treatment decisions. One potential application of Ki-67 is in the selection of patients for chemotherapy in addition to endocrine therapy. In patients with hormone receptor-positive tumors, a high Ki-67 expression may indicate a greater benefit from chemotherapy, as it suggests a more aggressive tumor with a higher risk of recurrence. However, the optimal cutoff value for Ki-67 to guide chemotherapy decisions remains to be determined. Another potential use of Ki-67 is in monitoring response to neoadjuvant chemotherapy. In the neoadjuvant setting, chemotherapy is given before surgery to shrink the tumor and improve the chances of successful surgery. Ki-67 expression can be measured before and after neoadjuvant chemotherapy to assess the response to treatment. A significant decrease in Ki-67 expression after chemotherapy may indicate a good response to treatment and a better prognosis. In addition to the factors mentioned above, it is important to emphasize the role of shared decision-making in breast cancer treatment. Shared decision-making is a process in which patients and clinicians work together to make treatment decisions that are consistent with the patient's values and preferences. In the context of HER2-negative breast cancer, shared decision-making is particularly important because there is often no single "best" treatment option. The optimal treatment approach may vary depending on the individual patient's circumstances and preferences. Clinicians should provide patients with clear and comprehensive information about the risks and benefits of different treatment options, including the potential impact on quality of life. Patients should be encouraged to ask questions and express their concerns. Together, patients and clinicians can make informed decisions that are aligned with the patient's goals and values. While the study did not find a significant association between Ki-67, HER2-negative status, and treatment outcomes, it is important to recognize that these factors may still play a role in treatment decision-making for specific subgroups of patients. For example, patients with hormone receptor-positive, HER2-negative tumors and high Ki-67 expression may benefit from the addition of chemotherapy to endocrine therapy. This is because high Ki-67 expression is associated with a more aggressive tumor phenotype and a higher risk of recurrence. Conversely, patients with hormone receptor-positive, HER2-negative tumors and low Ki-67 expression may be adequately treated with endocrine therapy alone, as these tumors are generally less aggressive and have a lower risk of recurrence. For patients with HER2-positive tumors, HER2-targeted therapies, such as trastuzumab, are the mainstay of treatment. However, the addition of chemotherapy to HER2-targeted therapy may be beneficial for patients with high-risk features, such as large tumor size, high grade, or lymph node involvement. HER2-negative breast cancer is a heterogeneous group of tumors with diverse biological and clinical characteristics. This heterogeneity presents challenges for treatment decision-making, as the optimal treatment approach may vary depending on the specific subtype and other factors such as tumor size, grade, and lymph node involvement. To address these challenges, researchers are working to identify specific molecular and genetic characteristics of HER2-negative tumors that may predict response to different therapies. This could lead to the development of more personalized treatment approaches that are tailored to the individual patient's tumor biology. Breast cancer treatment often involves a multidisciplinary team of healthcare professionals, including surgeons, medical oncologists, radiation oncologists, pathologists, radiologists, and nurses.

This multidisciplinary approach ensures that patients receive comprehensive and coordinated care that is tailored to their individual needs. In the context of HER2-negative breast cancer, the multidisciplinary team plays a crucial role in evaluating the patient's overall health status, assessing tumor characteristics, and developing a personalized treatment plan. The team also provides ongoing support and education to patients throughout their cancer journey.[18-20]

5. Conclusion

This study investigated the association of Ki-67 expression and HER2-negative status with tumor characteristics, treatment response, and disease progression in breast cancer patients. While Ki-67 and HER2-negative status are recognized as important prognostic factors in breast cancer, this study did not find a significant association with treatment modalities or tumor size in the studied population. The study revealed a predominance of Luminal B tumors, followed by Triple-Negative, Luminal A, and HER2-enriched subtypes. The majority of patients were HER2-negative (78.7%) and had a Ki-67 index >20% (77.7%), indicating a high proliferation rate among the tumor cells. No significant association was found between HER2-negative status and the type of therapy given (p=0.131) or tumor size (p=0.467). Similarly, Ki-67 expression >20% did not correlate significantly with the type of therapy (p=0.070) or tumor size (p=0.156). Further research with a larger sample size and longer follow-up is needed to validate these findings and explore the complex interplay of Ki-67, HER2 status, and other clinical variables in breast cancer progression.

6. References

1. Hacısalihoğlu UP, Dogan MA. Expression of estrogen and progesterone receptors, HER2 protein and Ki-67 proliferation index in breast carcinoma in both tumor tissue and tissue microarray. Biotech Histochem. 2022; 97(4): 298–305.
2. Brown J, Scardo S, Method M, Schlauch D, Misch A, Picard S, et al. A real-world retrospective study of the use of Ki-67 testing and treatment patterns in patients with HR+, HER2- early breast cancer in the United States. BMC Cancer. 2022; 22(1): 502.
3. Qi W-X, Chen L, Cao L, Xu C, Cai G, Chen J. Ki-67 index provides long-term survival information for early-stage HER2-low-positive breast cancer: a single-institute retrospective analysis. J Oncol. 2022; 2022: 4364151.
4. Satishkumar M, Ramesh M, Sanjive JG. A prospective multi-institutional observational study on ER, PR, HER2/NEU, and Ki-67 expression and discordance pattern in primary and metastatic and recurrent carcinoma breast and its therapeutic implications and prognostic outcome. Indian J Surg Oncol. 2023; 14(1): 72–80.
5. Hu X, Chen W, Li F, Ren P, Wu H, Zhang C, et al. Expression changes of ER, PR, HER2, and Ki-67 in primary and metastatic breast cancer and its clinical significance. Front Oncol. 2023; 13: 1053125.
6. Kalinin DV, Samoylova DV, Danilova NV, Germanovich NY, Kuznetsova OA, Zavalishina LE. Comparative immunohistochemistry study of different antibodies clones for detection of breast cancer markers (estrogen receptor, progesterone receptor, HER2/neu, Ki-67). Arkh Patol. 2024; 86(2): 14–20.
7. Kurbel S. Dependence of ki-67 values on tumor size in 1040 breast cancers of five immunohistochemical phenotypes: an argument for a minority of invasive breast cancers with high mitotic rates of a little dependence on ER and HER2 expressions. Int J Clin Stud Med Case Rep. 2024; 35(1).
8. Sharma S, Rayamajhi, Ghosh, Sharma, Sapkota. Study of impact of assessment of estrogen receptor, progesterone receptor, Her2 neu receptor and Ki-67 at diagnosis in management of breast cancer patient at Birat

Medical College Teaching Hospital (BMCTH). Birat J Health Sci. 2024; 9(1): 16–22.
9. Qayyum DZ. Immunohistochemical analysis of her2, ki-67, and p53 in various histological grades of breast carcinoma in local population. J Popul Ther Clin Pharmacol. 2024; 350–8.
10. Mohammed SS, Al Mahmoodi H, Yalda MI. Expression of Axl receptor tyrosine kinase and its association with Ki-67 proliferation marker, BCL-2 anti-apoptotic protein, hormone receptor status, and HER2/Neu status in Breast cancer among women from Duhok, Iraq. Cureus. 2024; 16(9): e70204.
11. Prediction of HER2 status in breast cancer patients based on DCE-MRI imaging features combined Ki-67 and VEGF expression. Eur J Gynaecol Oncol. 2025; 46(2): 71.
12. Liu L, Zong X-J, Wu J, Li J-J, Yuan P, Cao J. Utilizing multimodal ultrasonography in conjunction with serum Ki-67 and HER2 tests for breast cancer detection and prognosis. Clin Hemorheol Microcirc. 2025.
13. Crager M, Wijayawardana SR, Gruver AM, Blacklock A, Russell C, Baehner FL, et al. Population-based estimate for the correlation of the Oncotype Dx Breast Recurrence Score® result and Ki-67 IHC MIB-1 pharmDx in HR+, HER2-, node-positive early breast cancer. Breast Cancer Res. 2022; 24(1): 74.
14. Fouda M. Prognostic value of breast cancer subtypes based on ER/PR, HER2 expression and ki-67 index in women received adjuvant therapy after conservative surgery for early stages breast cancer a retrospective clinical study. Breast. 2018; 41: S20.
15. Ziegler C, Sotlar K, Hofmann DM, Kolben T, Harbeck N, Wuerstlein R. Use of the gene expression test prosigna® in premenopausal patients with HR+, HER2- early breast cancer: Correlation of the results with the proliferation marker Ki-67. Breast Care (Basel. 2024; 19(1): 34–42.
16. Arima N, Nishimura R, Osako T, Okumura Y, Nakano M, Fujisue M, et al. Ki-67 index value and progesterone receptor status can predict prognosis and suitable treatment in node-negative breast cancer patients with estrogen receptor-positive and HER2-negative tumors. Oncol Lett. 2018.
17. Kang Y-J, Lee H-B, Kim YG, Han J, Kim Y, Yoo T-K, et al. Ki-67 expression is a significant prognostic factor only when progesterone receptor expression is low in estrogen receptor-positive and HER2-negative early breast cancer. J Oncol. 2019; 2019: 7386734.
18. Ono M, Tsuda H, Yoshida M, Shimizu C, Kinoshita T, Tamura K. Prognostic significance of progesterone receptor expression in estrogen-receptor positive, HER2-negative, node-negative invasive breast cancer with a low ki-67 labeling index. Clin Breast Cancer. 2017; 17(1): 41–7.
19. Fasching PA, Gass P, Häberle L, Volz B, Hein A, Hack CC, et al. Prognostic effect of Ki-67 in common clinical subgroups of patients with HER2-negative, hormone receptor-positive early breast cancer. Breast Cancer Res Treat. 2019; 175(3): 617–25.
20. Mooghal M, Khan MAA, Samar MR, Shaikh H, Valimohammad AT, Idrees R, et al. Association between Ki-67 proliferative index and oncotype-Dx recurrence score in hormone receptor-positive, HER2-negative early breast cancers. a systematic review of the literature. Breast Cancer (Auckl). 2024; 18: 11782234241255211.