How Does Ki-67 Expression Vary Between Core Needle Biopsy and Surgical Specimens in Untreated Early-Stage Breast Cancer?

Oguzcan OZKAN[1], Asli GECGEL[1], Pinar PEKER[1], Gurdeniz SERIN[2], Osman ZEKIOGLU[2], Erhan GOKMEN[1]

[1] Ege University Faculty of Medicine, Department of Medical Oncology
[2] Ege University Faculty of Medicine, Department of Pathology

ABSTRACT

Ki-67 is a critical biomarker in early-stage breast cancer, influencing adjuvant treatment decisions in patients not receiving neoadjuvant therapy. Variations in Ki-67 between core needle biopsies (CNB) and postoperative specimens can complicate treatment planning. The aim of this study was to determine whether there is a significant difference in Ki-67 changes between CNB and surgical specimens in estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) positive and triple negative (TNBC) breast cancer subtypes. Data from 184 nonmetastatic, operable breast cancer patients who did not receive neoadjuvant treatment were analyzed. Age, tumor size, axillary lymph node involvement, adjuvant therapy, and CNB and postoperative Ki-67 values were evaluated. In the overall group, a statistically significant increase of 4.26 units and 2.50 units in the median was observed between pre and post-surgery Ki-67 values ($p< 0.05$). Significant increases were found in HER2-positive (mean change +7.58 ± 18.46%, 95% CI: 7.16–14.65, $p= 0.029$) and TNBC (mean change +14.58 ± 14.68%, 95% CI: 12.13–18.10, $p= 0.007$) subtypes, while the hormone receptor (HR)-positive group showed no significant median change (mean change +2.89 ± 10.89%, 95% CI: -1.58–2.43). In conclusion, HER2-positive and triple-negative tumors demonstrated a significant post-biopsy increase in Ki-67, indicating higher proliferative activity following biopsy. In HR-positive tumors, Ki-67 remained stable, indicating its reliability as a treatment predictor without genomic testing.

Keywords: Breast cancer, Ki-67 variability, Pathologic specimens, Adjuvant chemotherapy

INTRODUCTION

The immunohistochemical (IHC) detection of Ki-67-positive tumor cells serves as a crucial biomarker of cellular proliferation and has been integral to the clinical management of breast cancer for many years.[1] This labeling index is used to determine tumor aggressiveness because it allows measuring the proportion of actively dividing tumor cells.[2] In immunohistochemical evaluation, a technique commonly used to detect Ki-67 protein in tissue samples, the stained tissue is examined under a microscope, and the proportion of positively stained cells is manually calculated. The results of manual counting depend on the expertise of the pathologists. Interobserver variability can be high and lead to inconsistent results.[3,4] The adoption of digital measurement techniques for Ki-67 is recommended to enhance consistency and reliability in breast cancer assessments. Traditional visual scoring methods can be subjective and prone to interobserver variability, potentially impacting treatment decisions. Digital image analysis (DIA) offers a more standardized and reproducible approach.[5]

Furthermore, a study investigated the use of artificial intelligence (AI)-assisted methods for interpreting Ki-67 expression. The findings revealed that AI-assisted techniques demonstrated high interobserver agreement and closely matched gold standard values, indicating that AI has the potential to enhance the reproducibility and accuracy of Ki-67 assessments.[6]

In 2021, the International Ki-67 Working Group on Breast Cancer (IKWG) acknowledged the questionable analytical validity of Ki-67, proposed approaches to mitigate this limitation (e.g., preanalytical handling considerations, standardized visual scoring, participation in programs for quality assurance/control), and stated that Ki-67 ≤ 5% or ≥ 30% can be used to predict prognosis in T1-2, N0-1 ER-positive, HER2-negative patients.[7] Despite its prognostic value, Ki-67 assessment suffers from interobserver variability, especially in manually assessed specimens. The IKWG has proposed standardization efforts to improve its reproducibility in clinical practice.[8] In this patient group, high expression levels of Ki-67 often indicate more aggressive tumor biology and offer clues that greater benefit may be derived from intensified adjuvant therapies such as chemotherapy.[9] The use of cyclin-dependent kinase (CDK) inhibitors as adjuvant treatments in early-stage HR-positive breast cancer is influenced by specific histopathological factors, including tumor grade and Ki-67 levels.[10,11]

In contemporary clinical practice, genomic assays such as Oncotype DX, Prosigna, and Mammaprint have gained considerable traction, increasingly been into established clinical guidelines.[12] Prosigna and EndoPredict® are currently only validated at a prognostic level, while Oncotype DX, MammaPrint, and Breast Cancer Index (BCI) have both prognostic and predictive significance. However, the accessibility and financial constraints of these tests continue to pose challenges for many patients.[13]

Histopathological parameters such as ER, progesterone receptor (PR), HER2, Ki-67, tumor grade, and modified Bloom Richardson grade (MBRD) are essential in breast cancer diagnosis and treatment planning. However, discrepancies between diagnostic CNBs and postoperative surgical specimens can occur, potentially impacting clinical decisions.[14] Such variability in immunohistochemical markers may lead to undertreatment or overtreatment, depending on which specimen is used as the reference. This inconsistency is particularly pronounced in HR-positive breast cancer patients who do not undergo neoadjuvant therapy, leading to challenges in determining appropriate adjuvant treatments, such as chemotherapy and hormone therapy.[15] The decision-making process in treatment can vary significantly, as some authorities rely on CNB pathology while others prioritize operative specimen analysis.[1] A study analyzed Ki-67 indices in 89 pairs of CNBs and surgical specimens from invasive breast cancer patients without neoadjuvant therapy. Ki-67 was significantly higher in CNBs, with a median difference of 3.5%. Using a 14% cutoff, 18% of cases showed discrepancies, potentially affecting treatment decisions.[16] In another study, patients undergoing neoadjuvant chemotherapy exhibited a marked reduction in Ki-67 levels from a median of 28.6% in CNBs to 22.9% in surgical specimens indicating substantial changes posttreatment.[17] These challenges underscore the urgent need for standardized measurement protocols.

In light of these, our study aimed to investigate the changes in Ki-67 values between preoperative and postoperative samples across different molecular subtypes of breast cancer. By analyzing these variations, we sought to provide insights into the dynamic nature of tumor proliferation and its implications for the clinical utility of Ki-67 as a biomarker. Our findings contribute to the ongoing discourse on the standardization of Ki-67 assessment, particularly in the context of adjuvant treatment selection for HR-positive, HER2-negative, and high-risk breast cancer patients. This study aimed to elucidate variations in Ki-67 expression across immunohistochemical subtypes in early breast cancer patients not receiving neoadjuvant therapy.

PATIENTS AND METHODS

This retrospective study was conducted at Ege University Medical Faculty Hospital between January 2022 and August 2022. A total of 247 patients aged 18 years and older, diagnosed with invasive breast cancer, underwent total or partial mastectomy and were subsequently evaluated by the Breast Tumor

Council. Inclusion criteria were adult patients who underwent diagnostic CNB and mastectomy, with the operative specimens evaluated and reported by the same breast pathologist. Manual evaluation was performed using light microscopy by a single experienced breast pathologist.

Interobserver reliability could not be assessed, as all evaluations were performed by the same pathologist. Additionally, the pathologist was not blinded to previous Ki-67 values, which could introduce bias in assessments. Patients with male breast cancer, de novo metastatic breast cancer, or those who had received neoadjuvant chemotherapy or hormone therapy were excluded from the study. After excluding these patients, 184 patients remained eligible for inclusion.

All diagnostic and operative tissue samples were processed and analyzed by the same pathologist to ensure consistency in histopathological assessment. Ki-67 expression was determined manually by IHC. Specifically, tumor tissue samples were stained using a standard IHC protocol, and Ki-67 expression was evaluated by visual inspection under a light microscope. The pathologist examined the staining in tumor areas, counting the percentage of cells with nuclear staining, to establish the Ki-67 proliferation index. Staining was evaluated in at least five high power fields (HPFs) to ensure a reliable representation of tumor proliferation. Ki-67 was evaluated as a continuous variable. The Ki-67 score was calculated by averaging over five HPF instead of hot spot evaluation. Approximately 100 tumor cells were counted in each field and a total of approximately 500 cells per case were evaluated to determine the Ki-67 index. The Breast Pathologist's reports were retrospectively reviewed to confirm the consistency and accuracy of Ki-67 scoring, all of which were performed using manual assessment methods. Data collection, including patient demographics, clinical, histopathological, and treatment-related information, was conducted by accessing the Electronic Patient File system.

Ethics committee approval for the study was obtained from the Ege University Faculty of Medicine Ethics Committee (Decision No: 24-1.1T/36 Date: January 25, 2024).

Table 1. Clinicopathological characteristics of breast cancer patients: Tumor subtypes, size, and axillary involvement

Clinicopathologic characteristics of patients	n: 184 (%)
Histopathological features	
ER+/PR+ (HR-positive)	148 (80.4%)
HER2-positive	24 (13%)
Triple-negative	12 (6.5%)
Tumor size	
T1	104 (56.5%)
T2	59 (32%)
T3	21 (11.5%)
T4	0 (0%)
Axillary involvement	
Positive	55 (29.9%)
Negative	129 (70.1%)

Statistical Anaysis

The normality of Ki-67 value distributions was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests to evaluate conformity to a Gaussian distribution both preoperatively and postoperatively across groups, using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). The Wilcoxon signed-rank test was applied to determine the statistical significance of differences in Ki-67 values between these time points. Statistical significance was defined as a p-value of $p< 0.05$ with values below this threshold considered significant.

RESULTS

The clinicopathological characteristics of the 184 breast cancer patients included in the study are comprehensively summarized in Table 1. The cohort was stratified into three distinct molecular subtypes: HR-positive, HER2-positive, and TNBC. Notably, the HR-positive group represented the majority, comprising 148 patients (80.4%), highlighting its prevalence within the sample population. In contrast, the HER2-positive group consisted of 24 patients (13%), while the triple-negative group included 12 patients (6.5%), making up the smallest proportion of the total cohort. Eighty-five

Table 2. Ki-67 changes between CNB and operation specimens

		n	MedianKi-67 (range)%	MeanKi-67 (±SD)%	p
Overall		184			
	Baseline CNB		15.00 (0-80)	18.40 (±14.72)	
	Surgical specimen		17.50 (5 - 90)	22.66 (±16.24)	
	Change		0.00 (-45 - 60)	4.26	< 0.05
Hormone(ER/PR)positive		148			
	Baseline CNB		15.00 (0 - 80)	15.24 (±14.63)	
	Surgical specimen		15.00 (5 - 80)	18.13 (±15.35)	
	Change		0.00 (-20 - 35)	2.89 (±10.89)	< 0.001
HER2-positive		24			
	Baseline CNB		20.00 (5 - 80)	22.71 (±19.89)	
	Surgical specimen		25.00 (10 - 80)	30.29 (±19.95)	
	Change		5.00 (-45 - 60)	7.58 (±18.46)	0.029
Triple-negative		12			
	Baseline CNB		57.50 (10 - 80)	48.75 (±24.31)	
	Surgical specimen		65.00 (15 - 90)	63.33 (±21.56)	
	Change		10.00 (0 - 40)	14.58 (±14.68)	0.007

** Statistical comparisons were performed using the Wilcoxon Signed-Ranks Test*

patients (46.1%) underwent adjuvant chemotherapy. In the study cohort, no germline variants were detected in 58.7% of the patients, while genomic data were unavailable for 36.4%. In the remaining 4.9%, pathogenic or likely pathogenic variants were identified in genes such as BRCA1, BRCA2, CHEK2, PALB2, ATM, and RAD51C.

A detailed analysis of Ki-67 alterations is summarized in Table 2. In the overall patient group, the change in Ki-67 values was 0.00% (range: -45 to 60) as a median and 4.26% units as a mean. This change was found to be statistically significant ($p< 0.05$). In the HR-positive group, the median Ki-67 value demonstrated no change, remaining at 0.00 (range: -20 to 35), while the mean Ki-67 value increased by 2.89 units (±10.89). Despite the absence of a shift in the median, a statistically significant difference was observed between preoperative and postoperative Ki-67 values ($p< 0.05$), as revealed by the Wilcoxon signed-rank test. In contrast, the HER2-positive cohort exhibited a more marked increase in proliferation, with a median Ki-67 value rise of 5.00 units (range: -45 to 60) and a mean increase of 7.58 units (±18.46), which was statistically significant ($p= 0.029$). The TNBC group displayed the most pronounced changes, with a mean increase of 14.58 (±14.68) units and a median increase of 7.50 (0-40) units, both of which were statistically significant ($p= 0.007$). The Normality of the Ki-67 distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results indicated that the Ki-67 values in the HR-positive and HER2-positive groups did not follow a normal distribution at either the preoperative or postoperative time points. Conversely, the TNBC group demonstrated a normal distribution for Ki-67 values at both time points. To evaluate the statistical significance of changes in Ki-67 values pre and post-surgery, the nonparametric Wilcoxon signed-rank test was employed due to the lack of normal distribution in most subgroups. For the overall cohort, the Wilcoxon signed-rank test revealed a statistically significant difference in Ki-67 values before and after surgery ($p< 0.001$). While the mean Ki-67 value increased in the HR-positive group, the median remained unchanged, suggesting heterogeneity in individual responses rather than a uniform proliferative shift. In summary, there were no significant median changes in the hormone receptor (HR)-positive group (mean change +2.89 ± 10.89%, 95% CI: -1.58-2.43), but there were significant increases in the HER2-

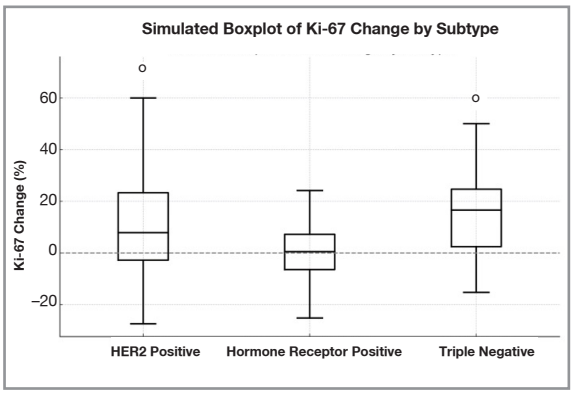

Figure 1. Mean Ki-67 changes in breast cancer subgroups. (Significant increases were found in HER2-positive (mean change +7.58 ± 18.46%, 95% CI: 7.16–14.65) and TNBC (mean change +14.58 ± 14.68%, 95% CI: 12.13–18.10) subtypes, while the hormone receptor (HR)-positive group showed no significant median change (mean change +2.89 ± 10.89%, 95% CI: -1.58–2.43).

positive (mean change +7.58 ± 18.46%, 95% CI: 7.16–14.65, p= 0.029) and TNBC (mean change +14.58 ± 14.68%, 95% CI: 12.13-18.10, p= 0.007) subtypes. Mean Ki-67 changes of the subgroups are presented in the box plot graph (Figure 1).

DISCUSSION

The Ki-67 index serves as a well-established prognostic and predictive marker in early breast cancer; however, there remains a scarcity of studies specifically investigating the prognostic implications of Ki-67 changes following CNB.[18] Adjuvant abemaciclib combined with endocrine therapy has demonstrated a substantial improvement in invasive disease-free survival (iDFS) and distant recurrence-free survival (DRFS) among patients with HR-positive, HER2-negative, node-positive, and high-risk early breast cancer, as evidenced in phase 3 monarchE trial. A pivotal aspect of this study was the utilization of Ki-67 as a biomarker to guide adjuvant treatment decisions, emphasizing its critical role in identifying patients at higher risk of recurrence.[19] Similarly, the NATALEE trial evaluated the efficacy and tolerability of 3 years of adjuvant ribociclib in combination with a non-steroidal aromatase inhibitor (NSAI) compared to NSAI alone in a broad population of patients with HR-positive, HER2-negative early breast cancer. A key finding of the study was the benefit observed in node-negative patients with a Ki-67 index of 20% or higher. This underscores the relevance of Ki-67 as a biomarker in guiding adjuvant therapy decisions, particularly for identifying high-risk patients who may derive significant benefit from ribociclib. In the NATALEE trial presented at the 2024 San Antonio Breast Cancer Symposium (SABCS), the 3-year iDFS results were reported as 90.7% (95% CI: 89.3% to 91.8%) versus 87.6% (95% CI: 86.1% to 88.9%). This benefit was observed irrespective of Ki-67 status.[20] The monarchE and NATALEE trials underscored the prognostic and predictive value of a high Ki-67 index, making it an essential criterion for tailoring adjuvant therapeutic strategies. However, a crucial unresolved question pertains to the selection of the appropriate sample whether to utilize Ki-67 measurements from preoperative core biopsy specimens or postoperative surgical samples. Preanalytical variables, such as tissue fixation and handling, can significantly influence Ki-67 results, thereby affecting clinical decision-making. Proper tissue handling is critical for accurate assessment of Ki-67 compared with ER and HER2. Attention to tissue fixation status is essential to ensure reliable Ki-67 evaluation.[21] Additionally, a systematic study examining the effects of delay to fixation (DTF) and time in fixative (TIF) on IHC using 24 cancer biomarkers found that differences in IHC staining were observed in formalin-fixed paraffin-embedded (FFPE) kidney tumor specimens after a DTF of ≥ 2 hours. Reductions in H-score and/or staining intensity were observed for several markers, indicating that preanalytical factors can compromise IHC results.[22]

Our study reveals that a significant proportion of patients exhibited increased Ki-67 expression levels after biopsy and that there are different patterns of change that can be observed across subtypes. In particular, patients with HER2-positive and triple-negative tumors showed a significant increase in Ki-67 levels. The HR-positive group represented the majority of the cohort, comprising 148 patients (80.4%), which underscores its prevalence within the sample population. This finding is consistent with existing literature, as HR-positive breast cancer is the most common subtype, accounting for approximately 70-80% of all breast cancer cases. These proportions are consistent with global epidemiological data. HER2-positive breast cancer is found in approximately 15-20% of cases, and

TNBC accounts for 10-15% of breast cancers.[23] HR-positive breast cancers generally have a better prognosis and are more likely to respond to endocrine therapies, making them a central focus of breast cancer research. The use of adjuvant chemotherapy is particularly prevalent in patients with larger tumors, lymph node involvement, or aggressive molecular subtypes, which are known to have a higher likelihood of recurrence and poor prognosis.[24] Adjuvant chemotherapy is commonly administered, particularly in HER2-positive and TNBC, which are considered aggressive tumor subtypes. These subtypes are associated with higher relapse rates and poorer overall survival, making adjuvant chemotherapy an important treatment strategy for high-risk patients.[25,26] The observed increase in Ki-67 post-biopsy in HER2-positive and TNBC subtypes suggests an adaptive proliferative response. Further large-scale, well-controlled clinical studies are needed to clarify whether changes in Ki-67 expression in these tumor subtypes should inform clinical decision-making.

In our study, although the mean Ki-67 score in the HR-positive group increased by 2.89 units between the preoperative and postoperative evaluations, there was no change in the median value. This result points to a common pattern of enhanced tumor cell proliferation after surgery. This indicates that while some tumors showed increased proliferative activity, others remained stable or decreased, resulting in an unchanged median. This heterogeneity likely reflects differences in tumor biology and the degree of sensitivity to procedural stress. Previous studies have reported similar findings, suggesting that HR-positive tumors are generally less responsive to surgical intervention in terms of proliferation rates compared to more aggressive subtypes, such as HER2-positive or TNBCs.[27] In these tumors, the role of hormone receptors in modulating tumor growth may limit the degree of change in proliferation observed after surgery.[28] The observed increase in Ki-67 following surgery in most cases may represent a short-term proliferative response to biopsy- or surgery-related inflammatory stimuli, again highlighting intra-group biological diversity. Moreover, the observed increase in mean Ki-67 values, although statistically significant, likely represents a transient response rather than a durable shift in tumor biology. Surgical trauma and the subsequent alteration of the tumor microenvironment can trigger a temporary increase in cell proliferation.[29] Despite the increase in mean Ki-67 in HR-positive tumors, the unchanged median suggests substantial heterogeneity and supports the reliability of preoperative Ki-67 values for clinical decision-making. Such findings reinforce the complexity of interpreting Ki-67 as a biomarker of tumor proliferation, as it is subject to various external factors, including treatment modalities and surgical interventions. The variability in individual responses underscores the importance of evaluating Ki-67 changes not solely based on central tendency measures but also considering individual trajectories and clinical context.

Ki-67 expression is correlated with S-phase and bromodeoxyuridine uptake, two additional indicators of proliferation. Although a high Ki-67 is indicative of a poor prognosis and a high likelihood of clinical response to chemotherapy, its independent relevance is low and it is not worth measuring in the majority of standard clinical situations.[30] Ki-67 was identified as an independent prognostic factor for disease-free survival in multivariate analysis studies using samples from randomized clinical trials with secondary central analysis of the biomarker (Hazard ratio 1.05-1.72) in a review by Luposi E., et al., assessing the prognostic and predictive value of Ki-67 in HR-positive patients and whether it can be used for treatment decision. Following chemotherapy, Ki-67 did not predict long-term follow-up. However, high Ki-67 was associated with immediate pathologic complete response in the neoadjuvant setting.[31] Previous research has yielded varied results on this subject, often confounded by the inclusion of patient groups undergoing neoadjuvant treatment, which can influence Ki-67 fluctuations.[32] Acs et al. emphasized the importance of prioritizing Ki-67 assessment on CNB specimens over excision samples in clinical decision-making to minimize the impact of preanalytical variables. They concluded that Ki-67 assessed by IHC should ideally be performed on CNB samples to most accurately reflect the biological status of the tumor. This approach reduces the influence of preanalytical factors, such as tissue handling and fixation, thereby ensuring more reliable and clinically meaningful results.[32]

Further supporting this perspective, a meta-analysis conducted by Kalvala et al. evaluated data from 5,982 patients who did not receive neoadjuvant therapy, examining the concordance of Ki-67 levels between CNB specimens and surgical samples across 22 studies. The meta-analysis reported a Cohen's kappa coefficient (κ) ranging from 0.261 to 0.712, indicating variable reliability, with agreement rates spanning from 70.3% to 92.7%.[33] Similarly, a study by Kim HS et al. demonstrated a high concordance rate between biopsy and surgical specimens. However, they also identified risk factors associated with discordance, particularly in patients with luminal subtype breast cancer. Of the 310 individuals in this research, 44 (14.2%) showed ΔKi-67 outliers (range: ≤ -20 or ≥ 28). Significant risk variables for ΔKi-67 outliers were found by multivariate analysis to be age ≤ 35 years, stage III malignancy, negative PR expression, and tumor size > 1 cm. Of the 171 patients with luminal HER2-negative tumors, 46 (26.9%) had a discordant breast cancer subtype based on preoperative or postoperative Ki-67 values, and a sizable fraction of these patients had at least one risk factor.[34]

Li et al. stratified 2858 HR-positive breast cancer patients who did not receive neoadjuvant therapy into low, intermediate, and high Ki-67 groups. Their analysis revealed that patients in the intermediate and high Ki-67 groups had significantly worse prognoses compared to those in the low Ki-67 group.[35] Based on these findings, the authors emphasized the critical importance of monitoring Ki-67 levels, as an indicator of tumor aggressiveness and potential recurrence risk. A separate study investigating patients who underwent neoadjuvant treatment reported significantly higher Ki-67 levels in CNB specimens compared to surgical samples.[16] Similarly, Gandini et al. analyzed 274 patients who did not receive neoadjuvant therapy and observed a notable increase in Ki-67 indices post-biopsy, with the most significant changes occurring in HER2-positive and triple-negative tumors.[36] Our findings align with these observations, highlighting the pronounced postoperative Ki-67 increases in HER2-positive and TNBC subtypes. This trend reflects the aggressive biology of these tumors and their heightened proliferative response to surgical or biopsy-related stress. In contrast, the HR-positive group showed a more modest increase in Ki-67 levels, with a stable median value, suggesting a more controlled tumor response likely influenced by HR activity.

In Summary, this study demonstrates significant increases in Ki-67 after CNB in HER2-positive and TNBC tumors, consistent with the literature. The clinical significance of this increase is critical. Increased Ki-67 for HER2-positive and TNBC patients may reflect increased tumor proliferation due to biopsy-related inflammatory responses. However, in HR-positive patients, Ki-67 is often used to guide chemotherapy decisions and CDK4/6 inhibitor eligibility. Discordant values (e.g., low Ki-67 at biopsy, high at surgery) may misguide treatment and lead to over- or undertreatment. In borderline cases, re-evaluation, consensus pathology, or the use of additional biomarkers may be warranted. In low- and middle-income countries (LMICs) where access to genomic testing is financially difficult, the use of Ki-67 in combination with other pathologic and clinical parameters may be recommended for HR-positive breast cancer in adjuvant chemotherapy and CDK4/6 inhibitor treatment decisions. The role of Ki-67 in CDK4/6 inhibitor eligibility is also important. Guidelines often use Ki-67 ≥ 20% as a threshold. Variability between CNB and surgical specimens may alter patient stratification and emphasize the need for consistent, reliable measurement. As we found, we believe that in patients who would qualify for adjuvant therapy and are not considering genomic testing, the current recommended systemic therapy can also be shifted to the neoadjuvant setting. Since Ki-67 did not show significant postoperative changes in this group of HR-positive patients, Ki-67 obtained from CNB appears to be a reliable parameter. Although consistent in this study (same pathologist), manual scoring is inherently subjective. The averaging method used in this study is less prone to exaggeration than hotspot methods. However, digital and AI-assisted scoring will further reduce variability. The high rate of unknown germline mutation status (n= 67, 36.4%) indicates that access to germline genetic tests is quite limited in our country and LMICs. During the period when the study was conducted, the germline testing rate in HR-positive patients in our clinic was quite low due to late test appointments and financial difficul-

ties. Our data show that access to both pathological genomic tests (such as Oncotype dx, Mammaprint) and germline NGS analyses is consistent with the world reality. In summary, our findings have practical importance in LMICs where genomic profiling is not always possible. In such settings, Ki-67 remains a valuable but limited biomarker. Standardizing its assessment is important to improve equity of care. Recent St. Gallen and ESMO guidelines recognize the prognostic importance of Ki-67 but warn against its use alone for treatment guidance.[37] Our findings support this stance and emphasize the need for integrative assessment approaches.

Limitations of study: Especially in the TNBC subgroup, the number of patients was small (n= 12), which limits the statistical power. Findings in this group should be interpreted with caution. In the study, no effect factor calculation was performed for subgroup analyses. On the other hand, manual Ki-67 assessment is subjective. Although all assessments were performed by the same pathologist using a mean method, inter-observer variability remains a limitation. The study is retrospective and despite ethical approval, inherent biases (e.g. missing data, selection bias) may be present. Mutation status was unknown in a significant proportion of patients, reflecting real-world constraints.

Conclusions

Our findings indicate that Ki-67 expression significantly increases post-biopsy in HER2-positive and TNBC tumors, potentially reflecting enhanced proliferative capacity. Although the mean Ki-67 value increased in HR-positive tumors, the unchanged median highlights intratumoral heterogeneity and reinforces the reliability of preoperative Ki-67 as a basis for clinical decision-making.

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Correspondence:

Dr. Oguzcan OZKAN

Ege Universitesi Tip Fakultesi

Onkoloji Anabilim Dali

Kazimdirik

35100 Bornova

IZMIR / TURKIYE

Tel: (+90-507) 042 40 30

e-mail: droguzcanozkan@yahoo.com

ORCIDs:

Oguzcan Ozkan	0000-0002-4075-7775
Asli Gecgel	0000-0001-6942-9858
Pinar Peker	0009-0004-8769-1584
Gurdeniz Serin	000-0003-4204-0601
Osman Zekioglu	000-0001-9728-4385
Erhan Gokmen	0000-0003-4596-2986