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RESEARCH ARTICLE

Ki-67 is a Prognostic Factor only With High Histological Grade and Low Progesterone-Receptor in early Estrogen-positive HER2-Negative Breast Cancer

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Abstract

Background: To evaluate the prognostic significance of Ki-67, histological grade, and progesterone receptors in patients with estrogen receptor-positive and human epidermal growth factor receptor 2-negative (HER2-) early breast cancer. Researchers have extensively examined tumor proliferative activity using the Ki-67 antigen over the past decade and have shown its value as a prognostic marker in breast cancer; however, its clinical utility remains debated. **Methods**: A retrospective study covering the period from 2016 to 2022 included 106 patients from the Brcko District with early estrogen-positive, HER2 receptor-negative breast cancer. The average age of the patients was 62.37±11.65 years. We divided the patients into groups based on high or low Ki-67 expression, high or low histological grade, and combined high or low Ki-67 expression with high or low progesterone receptor levels. We conducted multiple logistic regression analysis to evaluate factors related to disease relapse and mortality. **Results**: Multiple logistic regression analysis identified that the most significant prognostic factors for recurrence of early breast cancer with positive estrogen and negative HER2 receptors are high histological grade, especially when combined with high Ki-67 expression. The analysis also indicated that the key prognostic factors for mortality in these patients are high histological grade, elevated Ki-67 levels, and the combination of high histological grade with high Ki-67 expression. Low PR expression was associated with higher grade and elevated Ki-67. **Conclusion**: Ki-67 appears to have prognostic significance primarily when combined with high histological grade and low progesterone receptor expression, suggesting that this biomarker panel may help refine risk stratification in ER-positive, HER2-negative early breast cancer.

Keywords: Breast Neoplasms, Ki-67 Antigen, Receptors Progesterone, Neoplasm Grading

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Introduction

Ki-67 expression is strongly linked to aggressive tumor biology and tumor progression. Recognition has grown for Ki-67 as an excellent prognostic biomarker [1].

Researchers have recognized the Ki-67 index as a prognostic and predictive marker in luminal breast carcinoma for over a decade. However, the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) have not endorsed its routine use due to limited reproducibility [2- 4]. The clinical utility of Ki-67 as a prognostic marker may be more apparent when considered within more specifically defined tumor subgroups and/or as part of a multiparametric biomarker panel. For example, researchers developed a four-marker IHH-based test called IHC4, which includes estrogen receptor (ER), progesterone receptor (PgR), HER2, and Ki-67 [5]. Low-grade tumors showed low Ki-67 expression, while high-grade tumors exhibited high Ki-67 levels. Many previous studies [6, 7] have shown the link between Ki-67 and histological grade, but the results remain inconsistent, especially with the rapid emergence of other biological breast cancer markers. Several studies indicate that Ki-67 has prognostic value for recurrence and

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survival in patients with ER-positive and HER2-negative early breast cancer, but only within the context of low PgR expression levels [8, 9].

Clinicians have found Oncotype-Dx to be an essential tool for assessing the benefits of chemotherapy; however, its use remains limited in low- and middle-income countries. Due to a lack of access to this test, clinicians rely on traditional markers, such as the Ki-67 Proliferative Index, along with other clinicopathologic factors, to distinguish between low-risk and high-risk early breast cancer [10].

To our knowledge, there are no similar studies in our country, especially when considering the economic aspect, since available genomic assays are expensive. Our findings are particularly relevant in resource-limited settings, where access to genomic assays is restricted. In such contexts, traditional IHC biomarkers such as Ki-67, histological grade, and PR status represent a pragmatic and cost-effective approach for risk stratification. This study aims to show that the combination of high Ki67, low progesterone receptor, and high histological grade is a more accurate prognostic factor in breast cancer.

Mterials and Methods

Patient and study design

This retrospective study covered the period from 2016 to 2022 and included 106 patients from the Brcko District with early breast cancer, positive estrogen receptors, and negative HER2 receptors. Monitored parameters included patient clinical characteristics such as age, gender, menopausal status, histological grade of the tumor, tumor size, presence of metastases in the axillary lymph nodes, type of breast surgery, type of axillary surgery, introduction of neoadjuvant therapy, tumor biomarkers including estrogen and progesterone receptors, Ki-67, and HER2 receptors, as well as the presence of relapses (local or locoregional) and 5-year mortality. The study analyzed these 106 patients, dividing them into nine groups based on Ki-67 expression, histological grade, progesterone receptor expression, and neoadjuvant therapy.

We established the suspected diagnosis using radiological methods such as mammography, ultrasound, or magnetic resonance imaging, and confirmed it with a core biopsy before surgery. We included patients with a pathohistologically verified primary breast cancer, ensuring an age-balanced distribution with varying expression of the examined biomarkers. The exclusion criteria were breast cancers that are estrogen-negative, HER2-positive, in situ carcinoma, metastatic disease at initial diagnosis, prior cancer surgery, pathohistologically confirmed benign tumors, malignant non-carcinoma, distant metastases in breast tissue, or malignant comorbidities outside the breast, as well as inadequate samples with moderate to significant signs of necrosis or minimal tumor mass. We categorize recurrence into locoregional and distant types. Contralateral recurrences were not included in the recurrence category for this study. The primary endpoint was recurrence-free survival, specifically considering Ki-67 and PgR expression status and histological grade. The follow-up period spanned from surgery to the last hospital visit, regardless of department.

We divided the recurrence-free period into four groups based on its relationship with Ki-67 and PgR expression.

The secondary endpoint was overall survival, defined as the time from the date of surgery to the date of death or last follow-up. Mortality data were obtained from the Registry Office, with data collected up to the first month of 2023.

The study, approved by the ethics committee of the General Hospital of Brcko (number: 05EO-002/12), adhered to the highest ethical standards, ensuring the integrity and validity of our findings. The study followed ethical standards and described IHC methodology according to ASCO/CAP guidelines.

IHH determined the expression of ER, PgR, HER2, and Ki-67 in formalin-fixed, paraffin-embedded tissue blocks at the Department of Pathology of the Brcko District General Hospital.

Pathology stained Ki-67 following protocols provided by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). These guidelines include minimizing delays in prefixation, slicing surgical specimens into 5 to 10 mm pieces for fixation, and fixing in neutral buffered formalin for 6-72 hours [16]. They evaluated the expression levels of hormone receptors, HER2, and Ki-67 using the avidin-biotin complex technique. Tissues were sectioned into 4-μm-thick slices, deparaffinized with xylene, rehydrated through a graded ethanol series, and immersed in Tris-buffered saline. Then, they immunostained representative sections and examined more than 10 randomly selected fields per section under an optical microscope. After antigen retrieval, sections were incubated with primary antibodies against ER, PR, and HER2. The sections were then treated with a biotinylated anti-mouse secondary antibody and stained with streptavidin–horseradish peroxidase. The sections were counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted for microscopic analysis. The cutoff value used to distinguish low from high Ki-67 expression was the presence of Ki-67 immunoreactivity in more than 20% of stained nuclei in tumor tissues [11]. A PgR threshold of 20% was employed, as recommended by the 2013 International Breast Cancer Conference in St. Gallen. A low histological grade corresponds to grades I and II, while grade III indicates a high histological grade [12].

Statistical analysis

We used both non-parametric and parametric methods to determine statistical significance, and we assessed the distribution using the D'Agostino and Pearson omnibus test for normality. We applied Student's t-test, Mann-Whitney test, Fisher's test, and $\chi 2$ test to compare differences between groups. Additionally, we analyzed the risk factors related to disease relapse and mortality through univariate and multivariate logistic regression analyses to evaluate factors associated with disease relapse and mortality. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported. Statistical hypotheses were tested at a significance level of $\alpha = 0.05$, with differences

considered significant at $p < 0.05$. We marked statistical significance as $p < 0.05$, $p < 0.01$, and $p < 0.001$. All data were analyzed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA).

Results

The study involved 106 patients with early breast cancer who had positive estrogen receptors and negative HER2 receptors. The average age of all patients was 62.37±11.65 years. The youngest patient was 32, while the oldest was 86 years old.

In early breast cancer with positive estrogen and negative HER2 receptors, most patients exhibited significantly higher rates of Ki-67 negativity (2.8 times).

Compared to patients with low Ki-67 expression, those with high Ki-67 expression were significantly more likely to be postmenopausal, had a substantially higher histological grade, more often had tumors smaller than 2 cm, experienced more frequent metastases to the lymph nodes, underwent axillary surgery more frequently, and had a significantly higher mortality rate (Table 1).

Patients with early breast cancer who are positive for estrogen receptors and negative for HER2 receptors showed a lower histological grade. However, patients with higher histological grades were more likely to be postmenopausal, had more frequent metastases to the lymph nodes, higher occurrence of high Ki-67 expression, disease relapse, and a higher 5-year mortality rate (Table 2).

In early breast cancer with positive estrogen receptors and negative HER2 receptors, most patients showed significantly higher Pg positivity (1.7 times).

Compared to patients with high PgR expression, those with low PgR expression showed a significantly higher histological grade and Ki-67 expression, underwent mastectomy less often but partial breast resection more frequently, and experienced a substantially lower mortality rate (Table 3).

Univariate and multivariate logistic regression analyses showed that the most important prognostic factors for recurrence of early breast carcinoma with positive estrogen receptors and negative HER2 receptors are high histological grade, especially when combined with high Ki-67 expression. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported (Table 4).

Univariate and multivariate logistic regression analyses showed that the most important prognostic factors for mortality in early breast carcinoma with positive estrogen receptors and negative HER2 receptors are high histological grade, high Ki-67 expression, and the combination of high histological grade with high Ki-67 expression. Odds ratios (ORs) with 95% confidence intervals (CIs) were reported (Table 5).

Discussion

In this study, we confirmed the prognostic significance of Ki-67 expression and histological grade in patients with estrogen receptor (ER)-positive, HER2-negative early breast cancer. Our findings align with numerous previous reports that identify both high Ki-67 and high histological grade as independent predictors of poorer outcomes [13– 16]. Importantly, we demonstrated that combining these biomarkers enhances the accuracy of prognostic predictions compared to evaluating them separately, supporting earlier research that highlights the synergistic value of multiple markers [17– 20].

Our multiple linear regression analysis further identified high histological grade combined with elevated Ki-67 expression as significant predictors of disease recurrence and 5-year mortality. Previous studies suggest that integrating these factors into routine pathological evaluation improves risk stratification for patients and helps guide customized treatment decisions.

Regarding progesterone receptor (PR) status, the prognostic role remains complex. While an extensive cohort study by Lashen et al. (2023) showed that PR

Table 1. Clinical And Pathological Characteristics of Patients with Estrogen Receptor-Positive and HER2-Negative Early Breast Cancer Regarding Ki-67 Expression Levels

Monitored Parameter	High Ki-67		Low Ki-67		p-value
	N	%	N	%	
Number of patients	28	26.41%	78	73.59%	<0.0001*
Mean age [arithmetic mean ± SD] (years)	65.04 ± 7.71		61.41 ± 12.67		0.2418
Premenopausal	1	3.57	14	17.95	0.0306*
High histological grade	12	42.86	10	12.82	0.0004*
Tumor larger than 2 cm	20	71.43	35	44.87	0.0265*
Lymph node metastases present	16	57.14	29	37.18	0.0334*
Low Pg expression	18	64.29	21	26.92	0.0002*
Neoadjuvant therapy administered	2	7.14	15	19.23	0.0674
Mastectomy performed	18	64.29	38	48.72	0.0785
Breast-conserving surgery (partial resection)	10	35.71	40	51.28	0.0785
Axillary surgery performed	28	100	69	88.46	0.0301*
Recurrence present	4	14.29	9	11.54	0.3519
Death	2	7.14	0	0.00	0.0086*

Table 2. Clinical and Pathological Characteristics of Patients with Early Breast Cancer with Positive Estrogen and Negative HER2 Receptors about Histological Grade

Monitored Parameter	High Histological Grade		Low Histological Grade		p-value
	N	%	N	%	
Number of patients	22	20.76%	84	79.24%	<0.0001
Mean age [arithmetic mean ± SD] (years)	67.14 ± 7.95		61.12 ± 12.17		0.026*
Premenopausal	1	4.55	14	16.67	0.0332*
Tumor larger than 2 cm	9	40.91	42	50.00	0.2237
Lymph node metastases present	14	63.64	31	36.90	0.012*
High Ki-67 expression	12	54.55	16	19.05	0.0004*
Low Pg expression	16	72.73	23	27.38	<0.0001*
Neoadjuvant therapy administered	4	18.18	13	15.48	0.3791
Mastectomy performed	12	54.55	44	52.38	0.4282
Breast-conserving surgery (partial resection)	10	45.45	40	47.62	0.4282
Axillary surgery performed	20	90.91	77	91.67	0.4548
Recurrence present	6	27.27	7	8.33	0.008*
Death	2	9.09	0	0.00	0.0026*

negativity independently correlates with poorer survival, particularly in patients receiving endocrine therapy [21], our results indicate that low PR was associated with higher histological grade and increased Ki-67 expression, paradoxically, no deaths were observed in this subgroup. This likely reflects the limited sample size, treatment differences (e.g., higher rate of breast-conserving surgery), and relatively short follow-up rather than a true protective effect of low PR expression. Our findings align with Kang et al.'s (2019) observation that Ki-67's prognostic significance is heightened in cases of low PR expression [8].

Interestingly, Diana et al. (2022) reported that patients with low PR expression had higher histological grades and Ki-67 levels but exhibited lower mortality rates, which partially agrees with our finding that patients with low PR tended to undergo breast-conserving surgery more often and experienced better survival outcomes despite having more aggressive tumor features [22]. These observations imply that surgical treatment options and other clinical factors may influence survival independently of tumor biology.

Limitations of our study include a small sample size, a single-center design, and a short follow-up period. Additionally, the absence of molecular profiling beyond standard IHC markers restricts the ability to investigate the underlying tumor biology. Another important limitation is the absence of genomic assays such as OncotypeDx or MammaPrint, which prevents direct comparison with established molecular risk stratification tools. Larger, multi-institutional studies with extended follow-up are necessary to validate these results.

In conclusion, Ki-67 appears to have prognostic significance primarily when combined with high histological grade and low progesterone receptor expression, suggesting that this biomarker panel may help

Table 3. Clinical and Pathological Characteristics of Patients with Early Breast Cancer with Positive Estrogen Receptors and Negative HER2 Receptors Regarding Pg Expression Level.

Monitored Parameter	High Pg Expression		Low Pg Expression		p-value
	N	%	N	%	
Number of patients	67	63.21%	39	36.79%	<0.0001*
Mean age [arithmetic mean ± SD] (years)	61.1 ± 12.07		64.54 ± 10.69		0.1333
Premenopausal	11	16.42	4	10.26	0.1901
High histological grade	6	8.96	16	41.03	<0.0001*
Tumor larger than 2 cm	34	50.75	21	53.85	0.379
Lymph node metastases present	26	38.81	19	48.72	0.1597
High Ki-67 expression	10	14.93	18	46.15	0.0002*
Neoadjuvant therapy administered	11	16.42	6	15.38	0.4444
Mastectomy performed	44	65.67	12	30.77	0.0003*
Breast-conserving surgery (partial resection)	23	34.33	27	69.23	0.0003*
Axillary surgery performed	61	91.04	36	92.31	0.411
Recurrence present	8	11.94	5	12.82	0.447
Death	2	2.99	0	0.00	0.0086*

Table 4. Multiple Logistic Regression Analysis for Disease Recurrence

Factor	Disease Recurrence		
	OR	95% CI	p-value
Premenopausal	0.11	-0.09–0.35	0.2585
Histological grade	1.45	1.02–2.06	0.012*
Tumor >2 cm	0.96	0.83–1.12	0.2433
Lymph node metastases	1.07	0.93–1.24	0.3997
Ki-67 expression	1.02	0.86–1.21	0.7565
Pg expression	1.09	0.94–1.27	0.5246
$Ki\text{-}67^{+}$ / Pg^{-}	0.94	0.78–1.13	0.3128
$Ki\text{-}67^{+}$ / High Hist. Grade	1.03	0.88–1.21	<0.0001*

Table 5. Multiple Logistic Regression Analysis for Mortality

Factor	OR	95% CI	p-value
Premenopausal	0.40	0.07–0.80	0.4743
Histological grade	1.43	1.02–1.96	0.008*
Tumor >2 cm	0.96	0.82–1.12	0.2765
Lymph node metastases	1.16	0.91–1.49	0.2502
High Ki-67 expression	4.00	1.01–18.0	0.0281*
Progesterone receptor (-)	0.60	0.45–0.90	0.6001
$Ki\text{-}67^{+}$ / Pg^{-}	0.90	0.82–1.07	0.3703
$Ki\text{-}67^{+}$ / High Hist. Grade	4.00	1.01–17.0	0.0268*

refine risk stratification in ER-positive, HER2-negative early breast cancer. The clinical implication is that, in settings lacking access to genomic assays, combining Ki-67, histological grade, and PR status could help guide risk stratification and treatment decisions. Patients with the high-grade/high-Ki-67/low-PR profile may benefit from more aggressive adjuvant therapy. These results highlight the importance of integrated biomarker assessment to inform personalized treatment strategies and improve patient outcomes.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The Ethics Committee approved of the General Hospital of Brcko (No. 05EO-002/12).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Authors' Contributions

MP conceived and designed the study. MP collected the data and performed the analysis. MP wrote the manuscript. The author read and approved the final manuscript.

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