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Ki-67 and Histological Grade in Breast Cancer

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Abstract

AIMS: The aim of the study was to investigate the prognostic value of Ki-67 and histological grade in patients with estrogen receptor (ER) positive and human epidermal growth factor receptor 2 (HER2) negative early breast cancer. Although the proliferation activity of different tumors assessed with antigen Ki-67 was extensively studied in the last decade and showed that Ki67 expression was a useful prognostic factor in breast cancer, there is still a controversy about the utility value of Ki-67.

METHODS: The retrospective study covered the period from 2016 to 2022. Year included 106 patients from Brcko District, with early estrogen-positive and HER2 receptors-negative breast cancer. The average patients' age was 62.37 ± 11.65 years. Patients were divided into groups with high/low Ki-67 and high/low histological grade. Multiple linear regression analysis was performed for disease relapse and mortality.

RESULTS: Patients with high Ki-67 were more frequent postmenopausal, had higher histological grade, cancer <2 cm, more frequent lymph nodes' metastases, more frequently underwent to axillary surgery, and had a higher mortality rate. Patients with high histological grade were older, more frequent postmenopausal, had more frequent metastases to the lymph nodes, more frequent occurrence of high Ki-67, more frequent relapse of disease, and more frequent of death.

CONCLUSION: Combination of high Ki-67 with high histological grade in ER positive and HER2-negative early breast cancer is an important prognostic factor.

Introduction

Breast cancer is the most commonly diagnosed cancer of women worldwide with 2.26 million new cases in 2020 year [1]. Gene profiling identified four breast cancer subtypes (Luminal A, Luminal B, human epidermal growth factor receptor 2 [HER2] enriched and similar to basal), and immunohistochemistry (IHH) is used to determine these breast cancer subtypes. Luminal A is defined as positive for estrogen receptor (ER) and progesterone receptors (PgR), HER2 negative, Ki-67 "low" and risk of relapse "low" based on multiple gene expression test [2]. Although there is a collective recognition among the international guideline recommendations that Ki-67 is a prognostic biomarker in breast cancer, the international guideline recommendations for the use of Ki-67 in the prognostic and predictive evaluation of breast cancer remains are mixed [3]. Several international organizations, including the Italian Association of Medical Oncology, European Group on Tumor Markers, European Society for Medical Oncology, and National Institute for Health and Care Excellence, either recommend or consider Ki-67 for use in the prognostic evaluation of breast cancer patients, on the other hand, American Society of Clinical Oncology or the National Comprehensive Cancer Network does not support the use of Ki-67 in the prognostic or predictive evaluation of breast carcinoma. Only the European Society for Medical Oncology supports the use of Ki-67 expression as predictive of response to systemic chemotherapy, and only in the neoadjuvant setting [4].

The Ki-67 antigen, known as the Ki-67 proliferation marker (MKI67), is a human protein encoded with the MKI67 gene [5]. In 1986, Dr. Gerdes published the first paper on immunohistochemical determination of the growth fraction with Ki-67 in breast tissue, including breast cancer. The paper clearly showed that the expression of Ki-67 is significantly associated with the clinical degree of breast cancer [6]. Since that fundamental study, over 6,000 articles have

been published dealing with the use of Ki-67 in breast cancer (PubMed database November 2022). Despite of consistent data about Ki-67 index, the relationship between Ki-67 index and the other prognostic factors remains uncertain [7].

Multiple studies showed that histological grade has a prognostic value that is equivalent to lymph nodal status [9], and higher than the size of the tumor [10, 11]. Low-grade tumors showed low Ki-67 expression whereas high-grade tumors were presented with high Ki-67 expression. This association of Ki-67 and histological grade was demonstrated in many previous studies [12], [13], but it is still inconsistent, especially on the very rapid discovery of other biological markers of breast cancer.

Oncotype Dx has proven to be an essential tool in decision-making regarding the benefits of chemotherapy; its application is limited in low-middle-income countries. Due to a lack of ability to obtain this test, clinicians have resorted to reliance on traditional markers, like the Ki-67 proliferative Index in addition to the other clinic-pathologic factors to distinguish between the low-risk and high-risk early breast cancer [8].

To the best of our knowledge, there are no similar studies in our country, especially if the economic aspect is taken into account because available genomic assays are expensive so the aim of this study was to determine that the combination of Ki67 and histological grade are more accurate prognostic factors in breast cancer.

Patients and Methods

Patient and study design

This retrospective study covered the period from 2016 to 2022 year and included 106 patients from Brcko District, with early breast cancer, with positive estrogen and negative HER2 receptors. Monitored parameters were: Clinical characteristics of the patient: Age, gender, menopausal status, histological grade of the tumor, the size of the tumor, the presence of metastases in the axillary lymph nodes, type of breast surgery, type of operation on axils, introduction of neoadjuvant therapy, IHH tumor characteristics: ER, PgRs, Ki-67, HER2 receptors, the presence of relapses (local or locoregional), and 5-year mortality. Patients are divided into group with high/low Ki67 and high/low histological grade.

The suspected diagnosis was established by radiological methods (mammography, ultrasound, or magnetic resonance imaging) and after that confirmed by core biopsy before the operation. Including the criteria were: Pathohistologically verified primary breast cancer, age balance group with different expression of the examined biomarkers. Excluding the criteria were breast cancer that is estrogen negative, HER2 positivity, *in situ* carcinoma, metastatic disease at the initial diagnosis, previous cancer surgery, pathophysiologically confirmed diagnosis of benign tumor, malignant non-carcinoma, a distant metastatic process in breast tissue parenchyma or malignant comorbidity outside the breast, pathophysiologically inadequate sample with moderate to significant presence of signs of necrosis, and lower limit of tumor mass.

The study was approved by the Ethics Committee of general hospital of Brcko number: 05EO-002/12.

Methods

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

Ki-67 was painted according to protocols given by the American Association of Clinical Oncologists and the College of American Pathologists. These recommendations include minimizing the delay of pre-fixation, dividing surgical samples into slices of 5–10 mm for fixation, and fixation in neutral buffered formalin for 6–72 h [16]. Expression levels of hormone receptors, HER2, and Ki-67 were assessed using the avidin-biotin complex technique. Tissues were cut into 4-μm-thick sections, deparaffinized with xylene, rehydrated through a graded ethanol series, and immersed in tris-buffered saline. Representative sections were immunostained, and more than 10 visual fields were randomly selected and examined with an optical microscope. After antigen retrieval, the sections were incubated with primary antibodies against ER, PR, and HER2. Sections were then incubated with biotinylated anti-mouse secondary antibody and stained using streptavidin horseradish peroxidase. Sections were stained with Mayer's hematoxylin, dehydrated, cleared, and then mounted for examination. The limit value used to define the low to high expression of Ki-67 was the presence of Ki-67 immunoreactivity in more than 20% of colored nuclei in tumor tissues [14]. A low histological grade will imply histological Grades I and II, while a high histological grade will imply III [15].

Statistical analysis

Non-parametric and parametric methods are used to calculate statistical significance. The distribution value is determined D'Agostino and Pearson omnibus test normality. Student's t-test, Mann–Whitney test, Fisher's test, and χ^2 test were used for calculating the difference between the groups. The risk factors associated with disease relapse and mortality were assessed using multiple linear regression analysis. The statistical hypotheses were tested at the level of $\alpha = 0.05$, and the difference

between the groups in the sample was considered significant when $p < 0.05$ or less. Statistical significance was depicted as: $p < 0.05$, $p < 0.01$ and $p < 0.001$. All data were analyzed using GraphPad Prism version 8 (San Diego, California, USA).

Results

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

In early breast cancer with positive estrogen and negative HER2 receptors, most patients showed significantly more frequent Ki-67 negativity (2.8 times).

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

Table 1: Clinical and pathological characteristics of patients with early breast cancer with positive estrogen and negative HER2 receptors in relation to the height of expression of Ki-67

Monitored parameter	Ki-67 high		Ki-67 low		p-value
	n	%	n	%	
Number	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>2 cm	20	71.43	35	44.87	0.0265*
Metastases in the lymph nodes	16	57.14	29	37.18	0.0334*
Neoadjuvant therapy	2	7.14	15	19.23	0.0674
Mastectomy of the breast	18	64.29	38	48.72	0.0785
Partial resection of the breast	10	35.71	40	51.28	0.0785
Axillar surgery	28	100	69	88.46	0.0301*
Relapse present	4	14.29	9	11.54	0.3519
Fatal outcome	2	7.14	0	0.0	0.0086*

*The difference was statistically significant. SD: Standard deviation, HER2: Human epidermal growth factor receptor 2.

Patients with early breast cancer with positive estrogen and negative HER2 receptors showed more often low histological grade. However, patients with high histological grade were older than patients with low grade, were more often postmenopausal, and had more frequent metastases to the lymph nodes, occurrence of high Ki-67expression, relapse of diseased, and frequent 5-years mortality (Table 2).

Multiple linear regression analysis showed that the most important prognostic factors for early breast cancer relapse with positive estrogen and negative HER2 receptors were high histological grade, and especially the combination of high histological grade with high Ki-67 expression (Table 3).

At the same time, multiple linear regression analysis showed that the most important predictive factors for early breast cancer mortality with positive estrogen and negative HER2 receptors were high histological grade, high Ki-67 expression, and a combination of high histological grade with high Ki-67 expression (Table 4).

Table 2: Clinical and pathological characteristics of patients with early breast cancer with positive estrogen and negative HER2 receptors in relation to the height of histological grade

Monitored parameter	High histological grade		Low histological grade		p-value
	n	%	n	%	
Number	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≥2 cm	9	40.91	42	50.0	0.2237
Metastases in the lymph nodes	14	63.64	31	36.9	0.012*
High Ki-67 expression	12	54.55	16	19.05	0.0004*
Neoadjuvant therapy	4	18.18	13	15.48	0.3791
Mastectomy of the breast	12	54.55	44	52.38	0.4282
Partial resection of the breast	10	45.45	40	47.62	0.4282
Axillae surgery	20	90.91	77	91.67	0.4548
Relapse present	6	27.27	7	8.33	0.008*
Fatal outcome	2	9.09	0	0	0.0026*

*The difference was statistically significant. SD: Standard deviation, HER2: Human epidermal growth factor receptor 2.

Table 3: Multiple linear regression analysis for disease relapse

Monitored parameter	Relapse of the disease		
	OR	95% CI	p-value
Premenopausal	0.11	−0.09–0.35	0.2585
High histological grade	1.45	1.02–2.06	0.012*
Tumor>2 cm	0.96	0.83–1.12	0.2433
Metastases in the lymph nodes	1.07	0.93–1.24	0.3997
Ki-67 expression	1.02	0.86–1.21	0.7565
High Ki-67 expression with high histological grade	1.03	0.88–1.21	<0.0001*

*The difference was statistically significant. OR: Odds ratio, CI: Confidence interval.

Discussion

Although numerous studies to date have indicated the prognostic significance of high Ki-67 expression and high histological grade in breast cancer [12], [13], the results of individual studies are still inconsistent, probably due to the involvement of other biological mechanisms. However, several studies have also reported that high histological grade and expression Ki67 could accurately predict prognosis of breast cancer [17], [18]. Our study showed not only that Ki-67 and histological grade were strong prognostic factors for the breast cancer but also, more importantly, that the combination of these two factors should be used to more accurately predict the prognosis of ER-positive, HER2-negative early breast cancer patients [19].

Table 4: Multiple linear regression analysis for mortality

Monitored parameter	Mortality		
	OR	95% CI	p-value
Premenopausal	0.04	−0.07–0.08	0.4743
Height of histological grade	1.43	0.02–0.16	0.008*
Tumor>2 cm	0.96	−0.09–0.02	0.2765
Metastases in the lymph nodes	1.16	−0.02–0.09	0.2502
Ki-67 expression	0.04	0.01–0.18	0.0281*
High Ki-67 expression with high histological grade	0.04	0.01–0.17	0.0268*

*The difference was statistically significant. OR: Odds ratio, CI: Confidence interval.

Meta-analysis performed by Azambuja *et al*. published at British Journal of Cancer confirms that high Ki-67 expression in patients with early breast cancer confers worse prognosis in the overall population and quantifies its prognostic univariate impact.

Furthermore, it was also shown similar results in subgroup analyses for node-negative, node-positive, and untreated patients. This was the first meta-analysis of published studies to evaluate the association between Ki-67/MIB-1 expression and prognosis in early breast cancer [19].

On the other side, Alco *et al*. reported the results of a large study from Turkey in 2015 and revealed that the Ki-67 index was positively correlated with an increasing tumor size [20]. In our study, tumor size was similar in patients with low and high histological grade, as well as in patients with high and low Ki-67 expression. This can be partly explained by the relatively smaller sample of patients in our study, but also by the different biological profile of breast cancer in the study by Alco *et al*. and our study.

The axillary lymph node status is recognized as the most important determinant of breast cancer overall survival, and node-negative disease patients have a favorable prognosis and survival [15]. A number of studies reported that a higher Ki-67 index significantly correlated with positive lymph nodes [29], [30]. Our study showed more frequently metastases to the lymph nodes, and because that more frequently performed axillary surgery.

Study performed by Nishimukai *et al*. reported that high Ki-67 expression and low PgR expression could independently lead to a worse prognosis for postmenopausal patients with ER -positive and HER2-negative breast cancer [33]. Similar, our study showed that patients with high Ki-67 expression were more likely to be postmenopausal.

Our study had showed in early breast cancer with positive estrogen and negative HER2 receptors, most patients showed significantly more frequent Ki-67 negativity (2.8 times). Compared to patients with low Ki-67 expression, patients with high Ki-67 expression were more likely to be postmenopausal, had higher histological grade, more frequent cancer smaller than 2 cm, more frequent metastases to the lymph nodes, were more frequently performed axillary surgery and had a significant higher mortality rate. Similar results were obtained from the studies of other authors [21], [22], [23]. Hence, from this aspect of view, our results were consistent with the literature ones.

Large study with 161 708 cases conducted by Schwartz *et al*. reported that histologic grade continues to be of prognostic importance for overall survival despite tumor size and nodal status [24]. Rahka *et al*. in the study based on a large and well-characterized consecutive series of operable breast cancer in 2.219 cases reported also that histologic grade is strongly associated with both breast cancer-specific survival and disease-free survival in the whole series as well as in different subgroups based on tumor size (pT1a, pT1b, pT1c, and pT2) and LN stages (pN0 and pN1 and pN2) [25]. In our study patients with high histological grade had more frequent metastases to the lymph nodes, more frequent occurrence of high Ki-67 expression, and had significantly more frequent relapse of diseased and more frequent of death. Hence, also from this aspect, our results were consistent with the literature [25], [26].

Retrospective analysis evaluated the interaction between Ki-67 and histological grade and their prognostic role in different breast cancer subtypes performed by Liang *et al*. indicated that the trend of association between Ki-67 and relapse-free survival was parallel to the trend of association between histological grade and relapse-free survival in all patients and those within the different IHH based subtypes [17]. Both high Ki-67 expression and Grade 3 tumors were independent predictors of inferior relapse-free survival in all patients, especially in those with luminal-like tumors [17]. In our study, multiple linear regression analysis showed that the most important prognostic factors for early breast cancer relapse and 5-year mortality with positive estrogen and negative HER2 receptors are high histological grade, and especially the combination of high histological grade with high Ki-67 expression. Our results were not only consistent with the literature ones [27], [28], [29] but also showed that the combination of two prognostic factors can be more effective from the prognostic point of view than only one factor.

To the best of our knowledge, there are no similar studies in our country, especially if the economic aspect is taken into account, because available genomic assays are expensive so the present study not only showed that Ki-67 and histological grade are important and independent factors for the prognosis of breast cancer for luminal-like cancer, but also, more importantly, that these two factors can be used together to more accurately predict the prognosis of breast cancer patients.

Future research should focus on the standardization of Ki-67 assessment in routine clinical settings and on the role of Ki-67 in treatment decisions.

There are some limitations of the study, including limited sample size, relatively short follow-up time, and failure to investigate other potential molecular markers of breast cancer. Therefore, further studies on this topic are highly warranted.

Conclusion

The present study showed:

1. Ki-67 and histological grade are important and independent factors for the prognosis of breast

cancer for luminal-like cancer, but also, more importantly,

2. These two factors can be used together to more accurately predict the prognosis of breast cancer patients.

In future researhce should focus on the standardization of Ki-67 assessment in routine clinical settings and on the role of Ki-67 in treatment decisions.

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