ORIGINAL ARTICLE

Prognostic value of KI-67 proliferation index in luminal breast cancers

Valor pronóstico del índice de proliferación KI-67 en cánceres de mama luminales

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Abstract

Aim: *This study aimed to examine the prognostic significance of the KI-67 proliferation index, especially in breast cancer (BC) patients without HER-2 expression and no nodal involvement.* **Material and methods:** *The database of hormone-receptor-positive patients who underwent surgery for BC in our Surgical Oncology Clinic between 2008 and 2020 was retrospectively reviewed and recorded. Patients were categorized based on their KI-67 level, considering the cutoff value of 20%.* **Results:** *Our study revealed that tumors with high KI-67 levels were more likely to have a more advanced histological grade (p = 0.00) and size (p = 0.038). In the univariant analysis, KI-67 level was effective on overall survival (p = 0.044) and disease-free survival (p = 0.048). However, we found that there was no independent prognostic factor in the multivariant analysis.* **Conclusion:** *Although the Ki-67 proliferation index does not yet have an agreed threshold value and scoring methodology, it can also be used to determine prognosis and evaluate treatment response in some patients.*

Keywords: *Breast cancer. KI-67 index. Molecular subtypes. Prognostic factor.*

Resumen

Objetivo: *Este estudio tuvo como objetivo examinar la importancia pronóstica del índice de proliferación KI-67, especialmente en pacientes con cáncer de mama sin expresión de HER-2 y sin compromiso ganglionar.* **Material y métodos:** *Se revisó y registró retrospectivamente la base de datos de pacientes con receptores hormonales positivos intervenidas de cáncer de mama en nuestra Clínica de Oncología Quirúrgica entre 2008 y 2020. Las pacientes fueron categorizadas de acuerdo con su nivel de KI-67, considerando el valor de corte del 20%.* **Resultados:** *Nuestro estudio reveló que los tumores con valores elevados de KI-67 eran más propensos a tener un grado histológico (p = 0.00) y un tamaño (p = 0.038) más avanzados. En el análisis univariado, el nivel de KI-67 fue efectivo sobre la supervivencia global (p = 0.044) y la supervivencia libre de enfermedad (p = 0.048). Sin embargo, encontramos que no había ningún factor pronóstico independiente en el análisis multivariante.* **Conclusiones:** *Aunque el índice de proliferación Ki-67 aún no tiene un valor de umbral acordado ni una metodología de puntuación, también se puede utilizar para determinar el pronóstico y evaluar la respuesta al tratamiento en algunas pacientes.*

Palabras clave: *Cáncer de mama. Índice KI-67. Subtipos moleculares. Factor pronóstico.*

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Introduction

Prognostic factors are clinicopathological variables associated with outcomes (usually disease-free survival [DFS] and overall survival [OS]) used to predict the risk of death in post-surgical early breast cancer (BC). The KI-67 proliferation index has now been shown to be associated with poor clinical outcomes[1,2].

KI-67 is a non-histone type nuclear protein associated with cellular proliferation. It was first found by Gerdes et al. in the early 1980s using rat monoclonal antibodies in a cell line originating from Hodgkin lymphoma. Its function remains unclear, but it is thought to be involved in RNA synthesis. The most common analysis method of KI-67 antigen is detection by immunohistochemical (IHC) evaluation[3].

While KI-67 is expressed in the G1, S, G2, and M phases of the cell cycle, it is not detected in the G0 phase. MIB-1 is a monoclonal antibody developed against recombinant portions of the KI-67 antigen[1].

Proliferation is an important indicator used to predict BC prognosis and treatment response. The comparison of proliferation between uncontrolled growing tumor samples has become the most widely used method. Its potential uses include predicting resistance to chemotherapy or endocrine therapy, estimating residual risk in patients during standard therapy, and as a dynamic biomarker of treatment efficacy in samples taken at each stage in the neoadjuvant therapy process (especially neoadjuvant endocrine therapy). International researchers with significant expertise in evaluating KI-67 and the development of biomarker guidelines organize panels to make comprehensive recommendations on the interpretation and scoring of KI-67 based on the available evidence. Thus, they aim to achieve a consensus methodology, increase interlaboratory and interstudy comparability, and assist in its effective usability in clinical practice[4-6]. However, no consensus has been established on the issues listed yet. Expression of KI-67 has been associated with the luminal B phenotype, a high risk of recurrence, and a good response to neoadjuvant chemotherapy. Several guidelines emphasize the importance of KI-67 expression level in choosing those with early-stage BC and 1-3 positive axillary nodes not to administer adjuvant chemotherapy[7].

Although there is no standard threshold value for the KI-67 proliferation index and is not a standard in evaluation and scoring methodology, it is used to determine prognosis and other clinicopathological prognostic indicators.

In a meta-analysis of approximately 65,000 cases (41 studies), Petrelli et al. found that KI-67 had an independent prognostic value for OS in BC patients, for which only a cutoff value of > 25% was higher compared to lower rates and that it was associated with an increased risk of death[8].

It is now accepted that BC is not a single disease but a heterogeneous disease with biological diversity and molecular subtypes with different behaviors. One of the reasons for this heterogeneity is HER2 amplification[9]. Gene expression profiling has contributed significantly to our understanding of disease occurrence, progression, and recurrence. Therefore, it can be said that most of the clinical presentations of BC are determined within the molecular subtype profile[10].

Molecular subtypes are classified as luminal A, luminal B/HER2-, luminal B/HER2+, HER-2, and triple-negative according to the recommendations of the Gallen International Expert Consensus Report (2013)[11]. They approve a cutoff value of 14% KI-67 index in distinguishing luminal A and B subtypes. Cheang et al. found the best Ki-67 index cutoff point to be 13.25%[12].

The identification of BC molecular subtypes also helps us determine treatment options. While luminal tumors most likely respond to endocrine treatments such as tamoxifen, they are added to chemotherapy in addition to this treatment by their KI-67 levels. Tumors overexpressing HER2 respond favorably to treatment with trastuzumab in combination with an anthracycline taxane. On the other hand, triple-negative tumors have high genomic instability with an aggressive clinical course; therefore, treatment options are limited and non-specific[13-15].

There are significant differences in BC molecular subtypes in age, histological grade, lymph node status, and staging. Luminal type A BC is associated with the smallest tumor and the best prognosis. The HER2+ subtype is more commonly associated with a large tumor, positive lymph node metastasis, and poor grade[16]. Therefore, tumors with HER-2+ and axillary nodal metastases were not included in this study. We only aimed to determine the prognostic significance of the KI-67 index in luminal A and luminal B/HER2- tumors.

Material and method

Participants

Our study was initiated with the Medical Faculty Ethics Committee's approval (Decree number: İ2-123-21).

Patients with estrogen receptor (ER) and progesterone receptor (PR) positivity, no HER-2 expression, and no axillary lymph node involvement who were operated for BC in our Surgical Oncology Clinic between 2008 and 2020 were included in the study. Out of a total of 370 patients, 168 met the inclusion criteria. The hospital database was scanned retrospectively in a digital environment. Patients' age, menopausal status, and type of surgical procedure were extracted from the file contents and recorded. Besides, the following parameters were excluded from the histopathological reports: information such as receptor status (ER, PR, and HER-2), KI-67 percentage, histopathological subtype, size, Scarff-Bloom-Richardson (SBR) grade, lymphovascular invasion (LVI) status, and recurrence status were also recorded. Patients were categorized as over 14 and below according to their KI-67 percentage. For patients with recurrence, distant recurrences were defined as those occurring beyond the boundaries of the ipsilateral breast, chest wall, or regional lymph nodes. Distant recurrence sites were categorized as bone, brain, liver, lung, distant nodal, and multiple organ recurrence.

ER and PR status were determined using IHC staining. A positive ER or PR was considered when ≥ 1% of invading malignant cells display nuclear staining or immunoreactivity. Tumors were considered HER2-positive only if they showed 3+ staining with IHC staining or HER2 amplification (ratio > 2) using fluorescent *in situ* hybridization. ER, PR, and HER2 tests were scored per the American College of Pathologists Guidelines[17].

Histological grade was evaluated according to the Nottingham modification of the Bloom-Richardson system. Accordingly, grading was performed based on the Elston-Ellis modification by histochemical features such as tubular differentiation percentage, presence of nuclear atypia/pleomorphism, and the number of mitoses[17].

The patients were categorized into two groups by molecular BC subtypes according to the recommendations of the St. Gallen International Expert Consensus Report (2013) by molecular BC subtypes: According to the receptor status of their primary tumors, patients were categorized as follows: Luminal A (ER + and/or PR + and HER2-, Ki-67 < 14%); and luminal B/HER2- (ER + and/or PR +, HER2-, and Ki-67 ≥ 14%)[11].

DFS was determined as the time from diagnosis until the development of recurrence/metastasis or until the last follow-up date, and OS was the time from the date of diagnosis until death due to any cause. The follow-up period was the time from the date of diagnosis to the last follow-up date of the patient.

Post-operative adjuvant treatments of all patients were arranged according to NCCN guidelines. Radiotherapy was applied to the whole breast and tumor bed in patients who underwent breast-conserving surgery. Adjuvant chemotherapy with adjuvant endocrine therapy was applied to all pre-menopausal patients, Ki-67 > 10, tumor diameter > 5 mm, and high-grade tumors (Grade 2 and 3). Chemotherapy was neglected in postmenopausal patients with Ki-67 < 10, Grade 1, and ≤ 5 mm tumors.

Statistical analysis

Descriptive statistical analyzes of quantitative variables were performed and expressed as mean ± standard deviation, number, percentage, maximum and minimum values. Categorical variables were presented as frequency and percentage values. Survival curves were estimated using the Kaplan–Meier method, and the significance of the differences between these curves was determined using the log-rank test.

The relationship between categorical variables was analyzed using the chi-square (χ^2 test) test. Accordingly, KI-67 levels were compared with the following variables: status with menopause at diagnosis, operating tube, tumor size (T), histopathological subtype, histological grade, LVI status, and receptor status (ER and PR). Statistical analysis was performed at a 95% confidence interval. Univariant and multivariant analyzes were performed to identify independent prognostic factors affecting OS and DFS. The results were considered statistically significant if $p < 0.05$ (reported p-values were two-way.)

Results

All 168 patients included in the study were women. The mean age of the patients was 53.9 ± 12.4 years (30-92), and the mean follow-up period was 53 ± 31.8 months (1-117). By the menopausal status, 43.5% (n = 73) of the patients were pre-menopausal and 56.5% (n = 95) were post-menopausal. The affected breast was the right side in 53% (n = 89) of the patients and left in 47% (n = 79). The mean tumor size at the time of diagnosis was 17.5 ± 12.5 mm (2-80). Approximately ¾ of the patients had a tumor size of < 2 cm (n = 124.74%), while the remaining, except for five patients, had a tumor size of 2-5 cm.

Of the patients, 41% were luminal A (n = 69) and 59% luminal B HER- (p = 99). Most cases (n = 118, 70.2%) were ductal by histopathological subtypes, a few (n = 12, 20%) were lobular, and the remaining cases were of other histological types such as medullary tubular, metaplastic, adenoid, and papillary carcinoma. Most cancer cases determined were mildly differentiated (n = 65.39%), followed by moderately differentiated (n = 55.33%) and highly differentiated (n = 46.28%). The mean number of total lymph nodes excised was 9.2 ± 7 (1-30). Recurrence occurred in 4.8% of the patients (n = 8). Nine patients (5.4%) died. The Chi-square analysis p values showing that the clinicopathological features of the patients and their relationship with KI-67 levels are shown in table 1. The 1-, 2-, and 5-year OS and DFS rates determined with the survival analysis performed through the Kaplan–Meier method by the KI-67 levels are presented in table 2.

DFS in the group with Ki-67 < 20 (p = 0.048, 144.56 ± 1.64 months, 95% CI: 111.34-117.78) compared to the group with KI-67 ≥ 20 (p = 0.048, 101.03 ± 2.94 months, 95% CI: 95.26-106.80) it was long. Similarly, in the Ki-67 < 20 group, OS (p = 0.044, 114.25 ± 1.91 month, 95% CI: 110.50-117.99) KI-67 ≥ 20 (p = 0.044, 98.65 ± 3.27 month, 95% CI: 92.24-105.05) was also longer than the group with DFS and OS curves according to KI-67 levels are shown in figures 1 and 2.

The most common sites of metastases were isolated bone (n = 4, 44%), mixed type multiorgan metastases (n = 3, 34%), and local recurrence (n = 1, 12%), respectively. All of the mixed type metastases were liver metastases accompanied by bone metastases. The distribution of other clinicopathological features of the patients is shown in table 1. In the chi-square analysis between KI-67 levels and categorical variables, there was a significant correlation with tumor T stage (p = 0.038) and histological grade (p = 0.00). In contrast, no statistically significant relationship was found between LVI status, receptor status (ER, PR), histopathological subtype, the surgery type, and menopausal status (p < 0.05), (Table 1).

In the univariant analysis, PR (p = 0.047), grade (p = 0.02), and KI-67 levels (p = 0.048) were effective on DFS, while grade (p = 0.011), TNM stage (p = 0.024), and KI-67 levels were effective on OS. We found that the 67 level was effective (Table 3). However, in the multivariant analysis with these variables, we found that none of them were independent prognostic factors for PFS and OS (Table 4).

Table 1. Clinicopathological characteristics of 168 breast cancer patients by groups and p values of Chi-square analyzes of categorical variables

Variables	KI-67 < 20%	KI-67 ≥ 20%	Total	p value
	n = 92 (%)	n = 76 (%)	n = 168 (%)	
Surgery type				0.156
BCS	63 (68.5)	44 (57.9)	107 (63.7)	
Mastectomy	29 (31.5)	32 (42.1)	61 (36.3)	
Histology				0.985
Ductal	65 (70.7)	53 (69.7)	118 (70.2)	
Lobular	11 (12)	9 (11.8)	20 (12)	
Other	16 (17.3)	14 (18.4)	30 (17.8)	
T stage				0.038
T1 (< 2 cm)	73 (79.3)	51 (67.1)	124 (73.8)	
T2 (2-5 cm)	19 (20.7)	21 (27.6)	40 (23.8)	
T3 (> 5 cm)	0 (0)	4 (5.3)	4 (2.4)	
T4	0 (0)	0 (0)	0 (0)	
LVI status				0.462
Negative	80 (87)	63 (83)	143 (85)	
Positive	12 (13)	13 (17)	25 (15)	
Grade				0.00
Grade 1	42 (45.7)	14 (18.4)	56 (33.3)	
Grade 2	43 (46.7)	28 (36.8)	71 (42.3)	
Grade 3	7 (7.6)	34 (44.7)	41 (24.4)	
PR				0.145
Positive	86 (93.5)	66 (86.6)	152 (90.5)	
Negative	6 (6.5)	10 (13.2)	16 (9.5)	
ER				0.118
Positive	89 (96.7)	74 (97.4)	161 (95.8)	
Negative	3 (3.3)	2 (2.6)	5 (4.2)	
Menopause				0.537
No	38 (41.3)	35 (46.1)	73 (43.5)	
Yes	54 (58.7)	41 (53.9)	95 (56.5)	

BCS: breast conserving surgery; LVI: lymphovascular invasion.

Table 2. 1-2-5 year OS and DFS rates of the groups

Survival	KI-67 < 20% (%)			KI-67 ≥ 20% (%)		
	1	2	5	1	2	5
OS	98.8	97.4	95.7	96.9	95.3	93.4
DFS	100	98.5	96.1	98.5	95.5	93.4

DFS: disease free survival; OS: overall survival.

Adjuvant endocrine therapy was given to all patients who were scheduled for post-operative adjuvant therapy in accordance with the NCCN 2017 guideline. Only four of these patients received concomitant adjuvant chemotherapy. Therefore, univariant was not included in the analysis. Radiotherapy was applied to the entire breast and tumor bed in 107 patients who

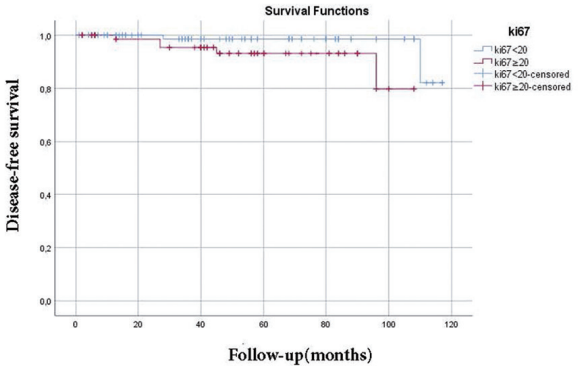

Figure 1. *Kaplan–Meier curve disease-free survival of KI-67 level (months).*

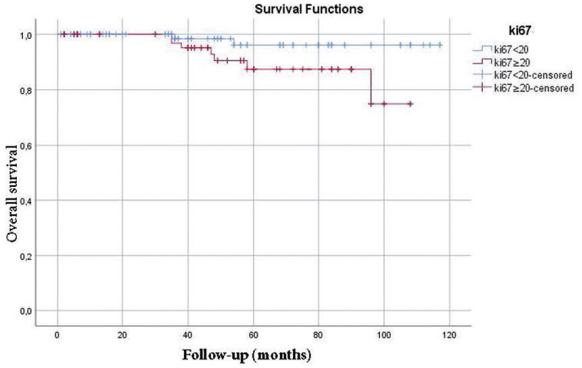

Figure 2. *Kaplan–Meier curve overall survival of KI-67 level (months).*

Table 3. Univariant analysis OS and DFS

	OS				DFS			
	p value	OR	95.0% CI		p value	OR	95.0% CI	
			Lower	Upper			Lower	Upper
Menopause	0.775	0.825	0.220	3.091	0.493	0.695	0.246	1.965
Age	0.425	0.973	0.911	1.040	0.274	0.955	0.880	1.037
Surgery type	0.324	1.384	0.726	2.637	0.170	1.861	0.767	4.514
Side	0.799	0.843	0.226	3.143	0.833	0.851	0.190	3.809
Histology	0.533	0.737	0.282	1.924	0.884	0.930	0.351	2.464
Tumor size	0.147	1.043	0.985	1.103	0.867	1.005	0.944	1.070
ER	0.334	0.355	0.044	2.897	0.762	21.414	0.000	-
PR	0.211	0.363	0.074	1.775	0.047	0.177	0.032	0.981
Luminal type	0.252	2.521	0.519	12.252	0.360	2.191	0.408	11.758
Grade	0.011	3.947	1.364	11.422	0.020	4.607	1.275	16.644
LVI	0.335	2.200	0.443	10.931	0.144	3.797	0.634	22.744
TLN	0.644	0.979	0.895	1,071	0.617	0.973	0.873	1.084
TNM stage	0.024	2.242	1.114	4,514	0.265	1.531	0.724	3.240
Radiotherapy	0.319	0.448	0.092	2.176	0.241	0.279	0.033	2.352
KI-67 level	0.044	4.207	10.37	20.395	0.048	6.456	1.013	55.856

CI: confidence interval; ER: estrogen receptor; LVI: lymphovascular invasion; OR: odds ratio; PR: progesterone receptor; DFS: disease free survival; OS: overall survival.

Table 4. Multivariant analysis OS and DFS

	OS				DFS			
	p value	OR	95.0% CI		p value	OR	95.0% CI	
			Lower	Upper			Lower	Upper
Grade	0.090	2.829	0.850	9.418	0.097	3.338	0.805	13.836
TNM stage	0.087	1.889	0.913	3.910	-	-	-	-
KI-67 level	0.630	1.573	0.249	9.921	0.466	2.432	0.223	26.484
PG	-	-	-	-	0.137	0.269	0.048	1.520

CI: confidence interval; LVI: lymphovascular invasion; OR: odds ratio; PR: progesterone receptor; DFS: disease free survival; OS: overall survival.

underwent breast-conserving surgery. In the univariant analysis, it was found that radiotherapy had no effect on OS (p = 0.319).

Discussion

In this study, the prognostic value of the KI-67 index and its relationship with other clinicopathological variables in luminal breast tumors (lumA, lumB HER-) without HER-2 negative and axillary lymph node involvement were examined. It was aimed to make the effect of the KI-67 index more evident by excluding patients with axillary nodal involvement and HER2 positive, which are considered to be one of the critical poor prognostic factors[18]. Our study revealed that tumors with high KI-67 levels were more likely to have more advanced histological grades and sizes (Table 1). In the survival analysis, we found that the 1-, 2-, and 5-year OS and DFS rates were better in the group with low KI-67 levels, and histological grade was vital among the factors affecting DFS (Table 2). In the univariant analysis, we found that grade, tumor size, and if the KI-67 level was above DFS, PR, grade, and KI-67 levels were effective on OS, but none of them were independent prognostic factors for OS and DFS in the multivariant analysis.

There is still no consensus on a standard cutoff value for KI-67. While a 14% cutoff point was recommended in the St Gallen consensus 2011 report, the 2015 report emphasized the labs' median values[11,19]. Various authors have researched this subject and expressed their opinions about the importance of different breakpoints. Petrelli et al. found a cutoff value of > 25% associated with a higher risk of death than lower expression rates, while a 10% value was associated with poor prognosis[8]. In two critical studies, the 20% cutoff point was more closely associated with poor outcomes[20,21]. In this study, the results were evaluated based on the 20% cut-off point.

St. Gallen International Consensus Guidelines 2021 panel discussed strategies for early BC treatment. According to the survey, the panel was unable to define a consistent Ki67 threshold of 10% to 25% to recommend chemotherapy in ER-positive, node-negative BC. Overall, the panel supported the recent working group recommendations that 5% of tumors with Ki67 did not receive chemotherapy, whereas tumors with Ki67 received 30% chemotherapy[22]. Panelists preferred that genomic signature testing be considered in the vast majority of cases in which chemotherapy is considered. In this, they referred to the importance of mature data to be collected from prospective studies (MINDACT, ADAPT, TAILORx, RxPonder, etc.) in which patients were classified based on well-established genomic signatures[23].

In clinical practice, early-stage BCs are classified into three subgroups according to their receptor expression status: ER- and/or PR-positive and HER2-negative, HER2-positive or triple-negative breast cancer (TNBC). The implications of these classifications for systemic therapy are as follows: almost all ER-positive tumors warrant adjuvant endocrine therapy, the majority of TNBCs warrant adjuvant chemotherapy, and most HER2-positive cancers warrant anti-HER2 therapy in combination with chemotherapy. Adjuvant endocrine therapy was given to all patients in the study due to ER expression. Adjuvant chemotherapy was given to the postmenopausal risk group (Grade2-3, Ki-67 > 10, tumor diameter > 5 mm) together with all premenopausal patients. Accordingly, only four patients were candidates for chemotherapy.

ER positive cancers are sometimes classified as 'luminal A-like' (low grade, low Ki-67, strong ER/PR expression) or 'luminal B-like' (high grade, higher Ki67, and lower levels of ER/PR expression)[11]. There is persistent debate about exact thresholds for Ki-67 to justify chemotherapy treatment. Because in reality, most of the early stage, ER-positive tumors fall between these accepted extremes (5-30%)[24].

While adjuvant treatments are being planned, we are in favor of approaching the threshold values of Ki-67 with care, especially in patients with a long life expectancy, until the heterogeneous nature of BC with many genomic signatures is clarified and its implications for treatment are revealed. In these patients, lower threshold values such as 5% should be preferred for the justification of chemotherapy. In elderly patients, the 20% cutoff value can be used to recommend chemotherapy. Because our study revealed that the aggressive features of BC become evident at Ki-67 levels above this value.

Voduc et al. identified a total of 325 local recurrences and 227 regional lymph node recurrences in their study, which included nearly 3000 tumors followed for 12 years. They found that luminal A tumors (ER or PR positive, HER2 negative, Ki-67-14%) had the best prognosis and the lowest local or regional recurrence rate[25].

A meta-analysis of 46 studies indicated that KI-67/MIB-1 positivity confers a higher risk of relapse and worse survival in patients with early BC[1].

In a study conducted, histological Grade I was associated with Luminal A, while Grade III was associated with Luminal B, and other subtypes[26]. Another study showed a significant association between KI-67 values above 20% and histological Grade 3, and these were associated with poor prognosis in early-stage BC patients without nodal involvement[27]. Similarly, in our study, Grade 1 tumors mainly were associated with luminal A subtype with KI-67 < 20%, reaching statistical significance, while others were more associated with Grade 2-3 tumors.

Centralized assessment of KI-67 in 10 collaborative studies (8088 patients) by Abubakar et al. was performed using an automated scoring protocol. The relationship between KI-67 levels and 10-year BC-specific survival (BCSS) was investigated. The KI-67 is divided into quarters with specific breakpoints. They found that patients in the highest quartile of KI-67 (> 12% positive KI-67 cells) had worse 10-year BCSS than patients in the lower three quartiles. This relationship was also statistically significant for ER-positive patients[28]. Small, low-grade tumors with low rates of axillary involvement are more likely to belong to the ER + PR ± HER2- group.[29] Bolat Küçükzeybek et al. reported a positive correlation of KI-67 proliferation index with histological grade and tumor size, as well as a poor effect at 7-year OS[30].

Luminal A subtype BC has also been associated with the smallest tumor and the best prognosis[16]. In this study, we found that in patients with CI-67 < 20%, tumors were mostly T1 (79.3%), and their association was significant, and OS and DFS rates were better than the others (Table 2 and Figs. 1 and 2).

Many studies in the literature have determined that the KI-67 proliferation index is associated with OS and DFS regardless of nodal status[1,20,31].

Metastases identified in our study were the most commonly isolated bone, followed by liver metastases accompanying bone involvement. Except for patients with widespread metastases, two main disease patterns in recurrent BC have been reported. Patients with ER +/PR + (luminal) tumors tend to develop more bone metastases but no brain metastases. The situation is the opposite in patients with ER–/PR–tnonluminal) tumors[32]. Clinically, the most common metastasis sites are organs such as bone, lung, central nervous system, and liver[33,34].

Conclusion

Besides the absence of a specific threshold value agreed on for the KI-67 proliferation index, a certain standard could not be determined in the evaluation and scoring methodology by the laboratories. Although the same antibodies are used in IHC staining, different laboratories may report different results that reach a statistically significant size due to this method difference. Even in the same patient sample, up to 25%, different results can be obtained[35]. Despite all these, it can be used to determine prognosis together with other prognostic indicators and evaluate treatment response in patients with HR-positive, HER-2 negative, and nodal involvement. There is a need for new classification methods based on IHC, genetic and molecular findings, as well as more randomized prospective studies for the standardization of evidence that will form the basis of these studies.

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Conflicts of interest

The authors declare no conflict of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained approval from the Ethics Committee for analysis and publication of routinely acquired clinical data and informed consent was not required for this retrospective observational study.

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