ORIGINAL ARTICLE

Correlation between numerical and categorical immunohistochemical score of Ki-67 and HER2 with clinicopathological parameters of breast cancer

Mirsad Dorić, Suada Kuskunović-Vlahovljak, Edina Lazović Salčin, Svjetlana Radović, Nina Čamdžić, Mirsad Babić, Haris Čampara

Department of Pathology, School of Medicine, University of Sarajevo, Bosnia and Herzegovina

ABSTRACT

Aim To evaluate the relationship between numerical and categorical immunohistochemical score of Ki-67 and human epidermal growth factor of receptor 2 (HER2) with clinicopathological parameters of breast cancer (BC).

Methods The study included 311 patients with invasive BC diagnosed at the Department of Pathology, School of Medicine in Sarajevo, Bosnia and Herzegovina, during the period 2015-2019. The expression level of Ki-67 and HER2 was detected by immunohistochemical analysis.

Results The expression of Ki-67, as a numerical variable correlated significantly with tumour grade (p=0.025), progesterone receptor (PR) (p=0.034) and categorical score of HER2 (p=0.028). When Ki-67 was categorized into high (>14%) and low (≤14%) level groups, a statistically significant association was found between Ki-67 level groups and HER2 status (categorical and numerical) (p=0.001 and p=0.043, respectively), as well as significant negative linear correlation with PR (p=0.037). The expression of HER2, as a numerical variable, showed a statistically significant correlation with tumour grade (p=0.038), PR (p=0.025) and categorical Ki-67 (p=0.043). Categorical score of HER2 correlated significantly with age (p=0.025), histologic type (p=0.039), tumour grade (p=0.016), estrogen receptor (ER), (p=0.002) progesterone receptor (PR) (p=0.0001), and categorical and numerical value of Ki-67 (p=0.0001 and p=0.0001, respectively).

Conclusion The results demonstrated that the categorical immunohistochemical score of HER2 provided a greater association with clinicopathological parameters than numerical score of BC. Furthermore, a slightly better correlation with clinicopathological parameters was shown by the numerical value than by the categorical score of Ki-67 by applying a cut-off value of 14%.

Key words: breast neoplasms, carcinoma, prognosis, proliferative activity

Corresponding author:
Mirsad Dorić
Department of Pathology,
School of Medicine,
University of Sarajevo
Čekaluša 90, 71000 Sarajevo,
Bosnia and Herzegovina
Phone: +387 33 666 964;
E-mail: mirsad.doric@mf.unsa.ba
ORCID ID: https://orcid.org/0000-0002-8595-2064

Original submission:
14 May 2020;
Revised submission:
16 June 2020;
Accepted:
23 July 2020
doi: 10.17392/1203-21

Med Glas (Zenica) 2021; 18(1):107-113

INTRODUCTION

Breast cancer (BC) is the most common cancer and the leading cause of death in women worldwide (1). The expression of biomarkers in BC is important to identify prognosis. Several classic BC markers, such as estrogen receptor (ER), progesterone receptor (PR), Ki-67 proliferative index, and human epidermal growth factor of receptor 2 (HER2) are relevant for therapeutic strategy and prognosis (2-4). In addition to these factors, the assessment of tumour proliferation pattern is important for a treatment decision (2-4).

Uncontrolled proliferation of tumour cells is a distinct feature of malignancy and can be assessed by various methods (5). Dowsett et al. found that the most commonly used measurement is immunohistochemical evaluation of Ki-67 antigen (6). Although Ki-67 is the most commonly used marker to evaluate proliferative index in BC, clearly defined cut-off values for high Ki-67 index have not been defined yet (7).

HER2 is an important regulator of the cell cycle, including cell proliferation, cell survival, and apoptosis (8,9). The amplification or overexpression of HER2 serves as a prognostic factor and also has a therapeutic significance (10,11).

The identification of the optimal method or methodologies for the assessment of proliferative activity in BC has been the subject of several previous studies (12-13). Despite numerous studies in this field, the relationship between the Ki-67 and other clinicopathological prognostic factors remains uncertain (14,15). The association of HER2 status and Ki-67 is still controversial, as some researchers have found a positive association with Ki-67, but others have not (14,16).

In routine clinical practice, the results of immunohistochemical analysis of Ki-67 and HER2 are presented as numerical and categorical values. Numerical score is used in determination of categorical score, which primarily has predictive significance but also is important in prognosis identification.

The aim of this study was to evaluate the relationship between numerical and categorical immunohistochemical score of Ki-67 and HER2 with clinicopathological characteristics of BC in order to determine which immunohistochemical score provides better prognostic significance.

PATIENTS AND METHODS

Patients and study design

A retrospective analysis was conducted using data of 311 patients with invasive BC diagnosed at the Department of Pathology, School of Medicine Sarajevo, Bosnia and Herzegovina, during the period from 2015 to 2019. All patients with BC underwent partial or total mastectomy with axillary lymph node dissection. No neoadjuvant chemotherapy or radiotherapy was administered before surgical treatment. Tissues specimens were fixed in 10% buffered formalin, paraffin embedded, processed, and stained with hematoxylin and eosin.

The study analysed patient's age, histologic tumour type according to the WHO (17); tumour size (using the TNM staging system) T1 (including T1a, T1b, and T1c) $\leq$2 cm, T2 >2 $\leq$ 5 cm, T3 >5 cm T4 (including T4a, T4b, T4c, and T4d) tumour of any size growing into the chest wall or skin (inflammatory breast cancer) (18); tumour grade (using Nottingham histological score, Elston and Ellis histologic grading criteria (19): grade I (well differentiated), grade II (moderately differentiated), and grade III (poorly differentiated); lymph node (LN) metastasis (negative, positive); and lymphatic vessel invasion (LVI) (absent, present).

Methods

Immunohistochemical analysis. 4-μm-thick sections of formalin-fixed, paraffin-embedded tissue were mounted on coated slides. The immunostained slides were examined for nuclear staining in the case of estrogen receptor (ER), progesterone receptor (PR), and Ki-67, and membrane staining in the case of HER2.

The primary antibody against the ER was performed in humidity chamber in EDTA buffer (pH 9) for 40 min (clone 1D5, Dako Cytomation, Glostrup, Denmark, dilution 1:30). The protocols for staining PR and Ki-67 included a microwave antigen retrieval step, 3 times for 5 minutes: anti-PR (clone PgR, Dako Cytomation, Glostrup, Denmark; dilution 1:30), anti-Ki-67 (clone MIB-1, Dako Cytomation, Glostrup, Denmark; dilution 1:10). Antigen retrieval for HER2 using HercepTest was performed following the manufacturer's protocol (Dako Cytomation).

Immunohistochemical evaluation. For hormone receptors, the proportion of positive staining tumour cells (expressed in percentage) and the average intensity of staining were evaluated based on Allred score method (20). Tumours were considered positive for ER and PR when at least 1% of the tumour cells showed unequivocal nuclear staining according the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) guidelines (21).

Interpretation of HER2 staining and scoring. HER2 was scored according to the pattern of membranous staining and percentage of stained malignant cells. HER2 staining was scored from 0 to 3+ (Hercep Test score) (according to the manufacturer) as follows: 0 - no staining or faint incomplete staining in <10% of cells; 1- faint incomplete staining in >10% of cells; 2 - weak to moderate complete staining in >10% of cells; 3 - strong complete staining in >10% of cells. In categorical scoring only score 3 was considered positive; if IHC is 0 or 1+, the tumour was considered HER2 negative; samples with HER2 score of 2+ was confirmed as HER2-negative or HER2-positive using chromogenic in situ hybridization (CISH).

Interpretation of Ki-67 staining and scoring. As Ki-67 is a nuclear protein, only nuclear staining (plus mitotic figures stained with Ki-67) was incorporated into the Ki-67 score. The fraction of proliferating cells was based on a count of at least 500 tumour cells. The Ki-67 proliferative index for each of the cases provided Ki-67 results using numerical and categorical scores. Numerical score in the range 0-100 corresponded to the percentage of positive tumour cells (the Ki-67 values were expressed as the percentage of positive cells in each case). In categorical score, cases with ≥ 14% positive nuclei were classified as positive (high) Ki-67 expression, and those with < 14% were classified as negative (low) Ki-67 expression.

Statistical analysis

Patients and clinicopathological characteristics were evaluated using descriptive statistics. The correlation between Ki-67 and HER2 as a categorical variable with other clinicopathologic parameters were evaluated using Pearson's χ^2 test and Spearman rank correlation test. Kruskal-Wallis or Mann-Whitney U test were used to evaluate the difference in the continuous variables between the mean score of Ki-67 and HER2 as a numerical variable with clinicopathologic factors. For all statistical analyses, a $p \leq 0.05$ was considered significant.

RESULTS

The present study was conducted on 311 breast cancer patients with the mean age of 60.65 ±11.25 years and age range of 32 to 89 years. Breast cancer was most common in postmenopausal women, 256 (82.3%). The majority of the tumours ranged between 2 and 5 cm in size (pT2), 167 (53.7%). Invasive ductal carcinoma was seen in 299 (73.6%), lobular carcinoma in 34 (10.9%) and other types in 48 (15.4%) patients (Table 1).

Table 1. Clinicopathological characteristics of 311 patients with breast cancer

Variables	Value
Mean age (years) (±SD)	60.65 ±11.25
Menopausal status (No, %)	
Premenopausal	55 (17.7)
Postmenopausal	256 (82.3)
Tumour size (AJCC) (No, %)	
pT1	116 (37.3)
pT2	167 (53.7)
pT3	16 (5.1)
pT4	12 (3.9)
Histological type (No, %)	
Ductal (NOS)	229 (73.6)
Lobular	34 (10.9)
Other	48 (15.4)
Nottingham grade (No, %)	
G1	49 (15.8)
G2	213 (68.5)
G3	49 (15.8)
ER status (No, %)	
Negative	75 (24.1)
Positive	236 (75.9)
PR status (No, %)	
Negative	91 (29.3)
Positive	220 (70.7)
HER-2 score (No, %)	
0	173 (55.6)
1 +	71 (22.8)
2 +	34 (10.9)
3 +	33 (10.6)
HER-2 status (No, %)	
Negative	257 (82.6)
Positive	54 (17.4)
Ki-67 (categorical) (No, %)	
Low (< 14%)	155 (49.8)
High (≥ 14%)	156 (50.2)
Mean Ki-67 (numerical) (±SD)	24.12±26.82
LN status (No, %)	
Negative	147 (47.3)
Positive	164 (52.7)
LVI (No, %)	
Absent	143 (46.0)
Present	168 (54.0)

AJCC, American Joint Committee on Cancer; NOS, not otherwise specified; ER, estrogen receptor, PR, progesterone receptor; HER, human epidermal growth factor; LN, lymph node status; LVI, lymphovascular invasion;

The numerical score of Ki-67 ranged from 1 to 95% (mean 24.12±26.82%). The expression of

Ki-67 as a numerical variable, showed a statistically significant correlation with tumour grade (p=0.025), PR (p=0.034), categorical score of HER2 (p=0.0001). There were no correlations between age, menopausal status, tumour size, LN status, LVI, ER, and categorical HER2 (p>0.05). However, 155 (out of 311; 49.8%) patients were in low, and 156 (50.2%) were in high Ki-67 expression group. High expression of Ki-67 was more frequent in the patients with high grade tumours, but without statistical significance (p =0.069), and showed correlation with HER2 status: categorical and numerical (p=0.001 and p=0.043, respectively). Also, there was a significant negative linear correlation with PR (p=0.041) (Table 2).

Table 2. Correlation of categorical and numerical scores of Ki-67 with clinicopathological characteristics of 311 patients with breast cancer

Variable	Categorical Ki-67		Numerical Ki-67	
	Correlation Coefficient	p	Correlation Coefficient	p
Age	-0.017	0.768	-0.051	0.337
Menopausal status	0.044	0.443	0.002	0.957
pT (tumour size)	0.001	0.981	-0.025	0.659
Histological type	-0.083	0.145	-0.100	0.078
Tumour grade	0.103	0.069	0.127	<0.025
LVI	-0.029	0.607	-0.055	0.333
LN status	-0.081	0.156	-0.077	0.174
ER	-0.081	0.155	-0.066	0.248
PR	-0.118	<0.037	-0.120	<0.034
HER2 categorical	0.219	<0.0001	0.240	<0.0001
HER2 numerical	0.115	<0.043	0.101	0.076

LVI, lymphovascular invasion; LN, lymph node status; ER, estrogen receptor; PR, progesterone receptor; HER, human epidermal growth factor;

The expression of HER2, as a numerical variable, showed statistically significant correlation with the tumour grade (p=0.038), PR (p=0.025) and categorical Ki-67 (p =0.043). No association was found between numerical score of HER2 and other clinicopathological parameters (Table 3).

Table 3. Correlation of categorical and numerical scores of human epidermal growth factor of receptor 2 (HER2) with clinicopathological parameters of 311 patients with breast cancer

Variable	Categorical HER2		Numerical HER2	
	Correlation Coefficient	p	Correlation Coefficient	p
Age	-0.127-	<0.025	-0.085	0.133
Menopausal status	-0.010	0.860	-0.011	0.848
pT (tumour size)	0.022	0.697	0.061	0.283
Histological type	-0.117	<0.039	-0.095	0.093
Tumour grade	0.136	<0.016	0.118	<0.038
LVI	0.014	0.804	0.035	0.544
LN	0.009	0.876	0.045	0.427
ER	-0.178	<0.002	-0.087	0.124
PR	-0.246	<0.0001	-0.127	<0.025
Ki-67 categorical	0.219	<0.0001	0.115	<0.043
Ki-67 numerical	0.240	<0.0001	0.101	0.076

HER, human epidermal growth factor; LVI, lymphovascular invasion; LN, lymph node status; ER, estrogen receptor; PR, progesterone receptor;

Categorical score of HER2 showed a statistically significant correlation with histologic type (p=0.039), tumour grade (p=0.016), ER (p=0.002), PR (p=0.0001), age (p=0.025), as well as with categorical and numerical value of Ki-67 (p=0.0001 and p=0.0001, respectively). No statistical differences in menopausal status, tumour size, LN status and LVI were observed (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study to compare the relationship between numerical and categorical immunohistochemical score of Ki-67 and HER2 with clinicopathological characteristics of breast cancer patients. However, the association of Ki-67 index with prognostic parameters of BC has been extensively studied (7,22,23). The correlation of Ki-67 with clinicopathologic factors varied, although the purpose of these studies was the assessment of prognosis and predictive value determination (4,24,25).

The results of this study showed a correlation between the expression of Ki-67 (as numerical variable) and tumour grade, PR, and numerical score of HER2. However, no correlation was observed between Ki-67 index and age, menopausal status, tumour size, histologic type, ER, LN status, LVI and categorical HER2.

Recent studies have shown that absolute (numerical) high expression of Ki-67 is associated with higher tumour size, higher LN status, higher tumour grade, ER/PR negativity, HER2 and LVI positivity (26,27). Our findings were not consistent with the results of previous studies. These discrepancies may be related to the patients and tumour heterogeneity. The mean Ki-67 score in the presented study was 24.31%, in contrast with results of Sun et al., with 31.22% (26).

When Ki-67 was categorized into high (>14%) and low (≤14%) level groups, a statistically significant association was revealed between Ki-67 expression and HER2 status (numerical and categorical), and significant negative linear correlation with PR. No significant correlation was observed with the rest of the clinicopathologic parameters.

A number of previous studies have investigated the correlation between Ki-67 and other clinicopathological parameters, using Ki-67 as a categorical variable (23, 27-29), however, the findings were controversial. The earliest study conducted

in the United Kingdom, demonstrated a significant association between the Ki-67 index and the histological grade, size and type of the tumours (30). A study that included a cohort of Pakistani patients revealed a significant association between Ki-67 expression and tumour grade, PR, HER2 and lymph node status (23). Alco et al. reported the results of the largest study from Turkey in 2015 and revealed that the Ki-67 index correlated positively with an increasing tumour size (28). In our study, an association was found between Ki-67 level (numerical and categorical) and tumour staging, but without statistical significance. This correlation was demonstrated in many previous studies (14, 27-27, 31). Consistent with the observations of other studies (32-34) our observation that Ki-67 positivity leads breast carcinoma in progression to higher histological grade, implies that Ki-67 high expression promotes tumour growth in breast cancer patients (34).

Several methods for assessing HER2 status are currently available, and each method has its proponents. Immunohistochemistry is the most frequently used, convenient and cost-effective initial test for HER2 protein expression. The results of immunohistochemistry are generally divided into four scale scores (range 0 to 3+), depending on the percentage of positive tumour cells and staining intensity (numerical score), then categorized into positive and negative (categorical score). HER-2 status is crucial in the guidance of treatment decisions for the use of trastuzumab and is becoming a standard recommendation in the pretreatment work-up of patients with invasive breast cancer (35). We did not find any data which investigate the correlation between numerical score of HER2 and clinicopathological parameters in literature except our results. We found that HER2 overexpression correlated negatively with PR expression, while correlating positively with the tumour grade and categorical Ki-67 positivity. No association was found between numerical score of HER2 and other clinicopathological parameters.

The overexpression or amplification of HER2 is an indicator of likelihood of response to anti-HER2 therapies (36). This is the predictive significance of HER2 overexpression (positivity). In all previous analyses, the prognostic value of HER2 was determined using a categorical result. In the presented study, HER2 overexpression was statistically significant with respect to age, histological type, tumour grade, ER, PR, categorical and numerical values of Ki-67. No statistical differences in menopausal status, tumour size, LN status and LVI were observed.

Numerous earlier researches have enrolled cases with HER-2 overexpression and reported their correlation with a high tumour grade, absence of ER or PR expression and high Ki-67 (31, 37-40). The coincidence of HER2 overexpression with Ki-67 high expression, PR and ER negativity indicates that there may be some regulatory relationship between HER2 and these genes in signal transduction pathways (39). Many studies have reported that age is an independent factor for poor prognosis in BC (41-43), which is consistent with the results of our study, but there are also studies that did not confirm these findings (44). Moreover, various studies have a lack of relationship between histologic type and HER2 status, which is inconsistent with our results (44,45).

In conclusion, the results of this study demonstrate that the categorical immunohistochemical score of HER2 provided a greater association with clinicopathological characteristics than numerical score of BC. This can be explained by equivocal HER2 +, which in the categorization is unequivocal and defined as positive or negative, by retesting using in situ hybridization methods. Also, there were small differences found between the correlation of numerical and categorical values of Ki-67 with clinicopathological parameters. A better association was shown by using the numerical value of Ki-67 than by using categorical score applying cut-off value of 14%. This indicates a necessity of new researches that would more precisely determine the cut-off value for Ki-67.

FUNDING

No specific funding was received for this study

TRANSPARENCY DECLARATION

Competing interests: None to declare.

REFERENCES

1. Siegel R, Jemal A. Cancer Facts and Figures. Atlanta: American Cancer Society, 2013.
2. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature 2000; 406:747-52.
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001; 98:10869–74.
4. Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, Zabaglo L, Mallon E, Green AR, Ellis IO, Howell A, Buzdar AU, Forbes JF. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. J Clin Oncol 2011; 29:4273–8.
5. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med 2016; 13:496-504.
6. Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, Ellis M, Henry NL, Hugh JC, Lively T, McShane L, Paik S, Penault-Llorca F, Prudkin L, Regan M, Salter J, Sotiriou C, Smith IE, Viale G, Zujewski JA, Hayes DF; International Ki-67 in Breast Cancer Working Group. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011; 103:1656-64.
7. Hashmi AA, Hashmi KA, Irfan M, Khan SM, Edhi MM, Ali JP, Hashmi SK, Asif H, Faridi N, Khan A. Ki67 index in intrinsic breast cancer subtypes and its association with prognostic parameters. BMC Res Notes 2019; 12:605.
8. Ménard S, Tagliabue E, Campiglio M, Pupa SM. Role of HER2 gene overexpression in breast carcinoma. J Cell Physiol 2000; 182:150–62.
9. Harari D, Yarden Y. Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. Oncogene 2000; 19:6102–14.
10. Lebeau A, Deimling D, Kaltz C, Sendelhofert A, Iff A, Luthardt B, Untch M, Löhrs U. Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. J Clin Oncol 2001; 19:354–63.
11. Varga Z, Noske A. Impact of modified 2013 ASCO/CAP guidelines on HER2 testing in breast Cancer. One Year Experience PLoS One 2015; 10:0140652.
12. Coates A. S, Winer E. P, Goldhirsch A, Gelber R. D, Gnant M, Piccart-Gebhart M, Thürlimann B, Senn H-J and Panel Members. Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Terapy of Early Breast Cancer. Ann Oncol 2015; 26:1533–46.
13. Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsky P, Loibl S, Colleoni M, Regan MM, Piccart-Gebhart M, Senn HJ, Thürlimann B, André F, Baselga J, Bergh J, Bonnefoi H, Brucker SY, Cardoso F, Carey L, Ciruelos E, Cuzick J, Denkert C, Di Leo A, Ejlertsen B, Francis P, Galimberti V, Garber J, Gulluoglu B, Goodwin P, Harbeck N, Hayes DF, Huang CS, Huober J, Khaled H, Jassem J, Jiang Z, Karlsson P, Morrow M, Orecchia R, Osborne KC, Pagani O, Partridge AH, Pritchard K, Ro J, Rutgers EJT, Sedlmayer F, Semiglazov V, Shao Z, Smith I, Toi M, Tutt A, Viale G, Watanabe T, Whelan TJ, Xu B. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Ann Oncol 2017; 28:1700-12.
14. Wiesner FG, Magener A, Fasching PA, Wesse J, Bani MR, Rauh C, Jud S, Schrauder M, Loehberg CR, Beckmann MW, Hartmann A, Lux MP. Ki-67 as a prognostic molecular marker in routine clinical use in breast cancer patients. Breast 2009; 18:135–41.
15. Tanei T, Shimomura A, Shimazu K, Nakayama T, Kim SJ, Iwamoto T, Tamaki Y, Noguchi S. Prognostic significance of Ki-67 index after neoadjuvant chemotherapy in breast cancer. Eur J Surg Oncol 2011; 37:155–61.
16. Bottini A, Berruti A, Bersiga A, Brizzi MP, Bruzzi P, Aguggini S, Brunelli A, Bolsi G, Allevi G, Generali D, Betri E, Bertoli G, Alquati P, Dogliotti L. Relationship between tumour shrinkage and reduction in Ki67 expression after primary chemotherapy in human breast cancer. Br J Cancer 2001; 85:1106–12.
17. Sinn HP, Kreipe H. A brief overview of the WHO classification of breast tumors, 4th edition, focusing on issues and updates from the 3rd Edition. Breast Care (Basel) 2013; 8:149-54.
18. Amin M B, Edge S, Greene F, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC, Jessup JM, Brierley JD, Gaspar LE, Schilsky RL, Balch CM, Winchester DP, Asare EA, Madera M, Gress DM, Meyer LR. AJCC Cancer Staging Manual. 8th ed. Chicago: Springer International Publishing AG, 2018.
19. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991; 19:403-10.
20. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 1987; 8:138–40.
21. Deyarmin B, Kane JL, Valente AL, van Laar R, Gallagher C, Shriver CD, Ellsworth RE. Effect of ASCO/CAP guidelines for determining ER status on molecular subtype. Ann Surg Oncol 2013: 20:87-93.

22. Luporsi E, André F, Spyratos F, Martin PM, Jacquemier J, Penault-Llorca F, Tubiana-Mathieu N, Sigal-Zafrani B, Arnould L, Gompel A, Egele C, Poulet B, Clough KB, Crouet H, Fourquet A, Lefranc JP, Mathelin C, Rouyer N, Serin D, Spielmann M, Haugh M, Chenard MP, Brain E, de Cremoux P, Bellocq JP. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Res Treat 2012; 132:895-915.
23. Haroon S, Hashmi AA, Khurshid A, Kanpurwala MA, Mujtuba S, Malik B, Faridi N. Ki67 index in breast cancer: correlation with other prognostic markers and potential in pakistani patients. Asian Pac J Cancer Prev 2013; 14:4353–58.
24. Denkert C, Loibl S, Müller BM, et al. Ki67 levels as predictive and prognostic parameters in pretherapeutic breast cancer core biopsies: a translational investigation in the neoadjuvant GeparTrio trial. Ann Oncol 2013; 24:2786–93.
25. Aman NA, Doukoure B, Koffi KD, Koui BS, Traore ZC, Kouyate M, Toure I, Effi AB. Immunohistochemical evaluation of Ki-67 and comparison with clinicopathologic factors in breast carcinomas. Asian Pac J Cancer Prev 2019; 20:73-9.
26. Sun J, Chen C, Wei W, Zheng H, Yuan J, Tu YI, Yao F, Wang L, Yao X, Li J, Li Y, Sun S. Associations and indications of Ki67 expression with clinicopathological parameters and molecular subtypes in invasive breast cancer: A population-based study. Oncol Lett 2015; 10:1741-48.
27. Kanyılmaz G, Yavuz BB, Aktan M, Karaağaç M, Uyar M, Fındık S. Prognostic importance of Ki-67 in breast cancer and its relationship with other prognostic factors. Eur J Breast Health 2019; 15:256-61.
28. Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Ordu C, Igdem S, Okkan S, Dincer M, Demir G, Ozmen V. Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 2015; 9:1046-54.
29. Kilickap S, Kaya Y, Yucel B, Tuncer E, Babacan Akgul N, Elagoz S. Higher Ki67 expression associates with unfavorable prognostic factors and shorter survival in breast cancer. Asian Pac J Cancer Prev 2014; 15:1381-85.
30. Pinder SE, Wencyk P, Sibbering DM, Bell JA, Elston CW, Nicholson R, Robertson JF, Blamey RW, Ellis IO. Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. Br J Cancer 1995; 71:146-9.
31. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, Ortmann O. Ki-67 is a prognostic parameter in breast cancer patients: results of large population-based cohort of a cancer registry. Breast Cancer Res Treat 2013; 139:539-52.
32. Zhou SJ, Guo H. Ki-67 expression and significance of different molecular subtypes of breast invasive ductal carcinoma. Zhonghua Yi Xue Za Zhi 2013; 93:2895-7.
33. Shapochka DO, Zaletok SP, Gnidyuk MI. Relationship between NF-kappaB, ER, PR, Her2/neu, Ki67, p53 expression in human breast cancer. Exp Oncol 2012; 34:358-63.
34. Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Exp Therapeutic Med 2010; 1:747-54.
35. Mendoza G, Portillo A, Olmos-Soto J. Accurate breast cancer diagnosis through real-time PCR her-2 gene quantification using immunohistochemically-identified biopsies. Oncol Lett 2013; 5:295-8.
36. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007; 25:118-45.
37. Shokouh TZ, Ezatollah A, Barand P. Interrelationship Between Ki67, HER2/neu, p53, ER, and PR Status and Their Associations with Tumor Grade and Lymph Node Involvement in Breast Carcinoma Subtypes. Medicine (Baltimore) 2015; 94:1359–64.
38. Liu Z, Zhang C, Zhuo P, He K, Wang X, Yu Q, Huo Z, Wang F, Yu Z. Characteristic of ER+/PR- and Ki67 value with breast cancer. Int J Clin Exp Med 2017; 10:3533-9.
39. Ding L, Zhang Z, Xu Y, Zhang Y. Comparative study of Her-2, p53, Ki-67 expression and clinicopathological characteristics of breast cancer in a cohort of northern China female patients. Bioengineered 2017; 8:383-92.
40. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med 2016; 13:496-504.
41. AlZaman AS, Mughal SA, AlZaman YS, AlZaman ES. Correlation between hormone receptor status and age, and its prognostic implications in breast cancer patients in Bahrain. Saudi Med J 2016; 37:37-42.
42. Lobbezoo DJ, van Kampen RJ, Voogd AC, Dercksen MW, van den Berkmortel F, Smilde TJ, van de Wouw AJ, Peters FP, van Riel JM, Peters NA, de Boer M, Borm GF, Tjan-Heijnen VC. Prognosis of metastatic breast cancer subtypes: the hormone receptor/HER2-positive subtype is associated with the most favorable outcome. Breast Cancer Res Treat 2013; 141:507-14.
43. Gabriel CA, Domchek SM. Breast cancer in young women. Breast Cancer Res 2010; 12:212.
44. Liu X, Zheng Y, Qiao C, Qv F, Wang J, Ding B, Sun Y, Wang Y. Expression of SATB1 and HER2 in breast cancer and the correlations with clinicopathologic characteristics. Diagn Pathol 2015; 10:50.
45. Aman NA, Doukoure B, Koffi KD, Koui BS, Traore ZC, Kouyate M, Effi AB. HER2 overexpression and correlation with other significant clinicopathologic parameters in Ivorian breast cancer women. BMC Clin Pathol 2019; 17:19:1.