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ORIGINAL ARTICLE

Importance of Ki-67 in human epidermal growth factor receptor 2 positive breast cancer

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Summary

Purpose: *The aim of this study was to evaluate the importance of Ki-67 in Human Epidermal Growth Factor Receptor 2 (Her-2) positive breast cancer patients.*

Methods: *We reviewed the records of patients diagnosed with Her-2-positive non-metastatic breast cancer between 2005 and 2011. Paraffin-embedded tissue samples were stained with MIB-1 mouse monoclonal antibody to find Ki-67 levels. Patients were grouped as low Ki-67 <20% and high Ki-67 ≥20%. Demographic and clinical features were compared.*

Results: *One hundred and six patients were included in the study. Median follow up time was 41 months (range 15-100). Median age was 49.5 years (range 29-79). Twenty-nine patients (27.4%) were in the Ki-67 low group. Demographic features were similar in both groups. Lymphovascular invasion was more frequent in the Ki-67 high group, and hormone receptor (HR) positivity was more frequent in the Ki-67 low group (p=0.03, p=0.03, respectively). Recurrence rate was not significantly different in both groups (p=0.36). T stage (p=0.02), stage (p<0.01), lymphovascular invasion (p=0.02), ER status (p=0.02), and HR status (p<0.01) were related with recurrence. In multivariate analysis, stage and HR negativity were independent factors for recurrence (p<0.01, p=0.01, respectively). Recurrence sites were also similar in both groups. Survival rates at the third year for Ki-67 low group and Ki-67 high group were 94% and 92%, respectively.*

Conclusion: *Her-2 positive patients with low Ki-67 and high Ki-67 had similar demographic and pathologic features except lymphovascular invasion and HR status. HR status was an important factor for disease course. Clinical course was determined by HR status rather than Ki-67.*

Key words: *breast cancer, Her-2, Ki-67, lymphovascular invasion*

Introduction

Breast cancer is the most common cancer and the most common cause of death due to cancer among females [1]. As a result, it is one of the cancers most studied. Depending on the advances in molecular biology, knowledge about breast cancer and its management have changed with time. Identification of Her-2 gene amplification or protein overexpression in breast cancer cells and the discovery of drugs that block this receptor have changed clinical practice. Her-2 positivity is related with the absence of steroid receptors, higher tumor grade, aneuploidy and high proliferation rate. Therefore, the prognosis of patients with Her-2 positive breast cancer is generally poor [2].

Gerdes et al. [3] defined a non-histone nuclear protein that increases in mitosis, named Ki-67. Studies suggest that Ki-67 plays its role in

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the early phases of RNA synthesis; however, the exact role of Ki-67 is not clear yet [4]. Ki-67 level increases during G1, S, G2 and mitosis phases, after division declines [5]. Ki-67 is closely related with proliferation, also called as "proliferation index". Proliferation index or Ki-67 is expressed as the percentage of MIB1 (a Ki-67 antibody) stained cells. In other words, it is the ratio of proliferating malignant cells. Therefore, it reflects the proliferative capacity of the tumor and is a prognostic factor for breast cancer [6].

Despite similar histopathologic findings, different molecular subtypes of breast cancer that have different prognosis and clinical properties have been defined by genetic array testing [7,8]. We know that luminal B breast cancer that has high proliferation index (Ki-67 ≥ 14%) has poorer prognosis than its counterpart Luminal A type that has low proliferation index (Ki-67 <14) [9]. The prognostic significance of Ki-67 in breast cancer is clearly defined in luminal subtypes. Her-2 positive tumors have higher Ki-67 levels than negative tumors [10].

The purpose of this study was to define the demographic, pathologic, and clinical properties of Her-2 positive breast cancer in relation with Ki-67 and define the importance of Ki-67 in Her-2 positive patients.

Methods

We retrospectively reviewed the records of patients diagnosed with Her-2 positive breast cancer between 2005 and 2011 at Trakya University, Medical Oncology Department. This study was approved by the Institutional Review Board. Her-2 positivity was defined as 3+ staining with immunohistochemistry (IHC) or presence of Her-2/neu amplification on *in situ* hybridization in case of 2+ staining. We collected paraffin-embedded tissue blocks of patients from the archive of our pathology department. Samples were stained with MIB1 mouse monoclonal antibody. The immunohistochemical staining was evaluated by an experienced pathologist. To find out Ki-67 levels a total of 2000 cancer cells were counted, and the proportion of MIB1 stained cancer cells to total number as percentage was calculated. Patients were grouped as Ki-67 low (<20%) and Ki-67 high (≥20%).

Factors retrieved from patient records included demographic factors, i.e. age, age at menarche, age at menopause, menopausal status, number of children, body-mass index and use of hormone replacement therapy; treatment related factors included type of surgery, data about chemotherapy, radiotherapy, hormonotherapy; recurrence related factors included date of recurrence and recurrence sites. The histopathological data, including tumor diameter, hormone receptor status, lymphovascular invasion, axillary lymph node involvement, and tumor grade (according to Scharf–Bloom–Richardson grading), were obtained from the pathology reports. Staging of the tumor was based on the American Joint Committee on Cancer (7th Edn) [11].

Statistics

Demographic and clinical features of low and high Ki-67 groups were compared. Non-parametric variables were studied by x^2 test. Parametric variables were compared with independent-sample t-test. Multivariate logistic regression analysis was performed to determine the predictive factors related with recurrence. A p value <0.05 was considered statistically significant.

Results

One hundred and sixty-five Her-2 positive non-metastatic patients at admission were determined. One hundred and six patients whom paraffin embedded tissue samples were present in the pathology archive were included in this study. Median follow up time was 41 months (range 15-100). Twenty-nine patients (27.4%) were in the Ki-67 low group, and 77 (72.6%) in the Ki-67 high group.

Median age was 49.5 years (range 29-79). Median age at menarche was 13 years (range 11–17). Forty nine (46.2%) of the patients were premenopausal. Only 7 patients were using hormone replacement therapy at the time of breast cancer diagnosis. The mean body mass index of the patients was 28.7±5.1 kg/m^2. Demographic characteristics of patients in both groups did not differ (Table 1).

The median Ki-67 was 30% (range 5-90) in the whole patient population. The median tumor diameter was 3 cm (range 0.5–10). The median number of dissected lymph nodes was 13 (range 1–46) and the median number of involved lymph nodes was 1 (range 0–31). Lymphovascular invasion was present in 49 (63.4%) of the patients; lymphovascular invasion was significantly more frequent in the Ki-67 high group (p=0.03). Seventy-one patients (66.9%) were HR positive. Hormone receptor and ER positivity were more frequent in patients with low Ki-67 (p=0.03, p=0.04, respectively). Other pathologic characteristics of the tumors were not significantly different (Table 2).

All patients except one with inflammatory breast cancer were subjected to surgery. Breast conserving surgery was performed to 31 pa-

Table 1. Comparison of demographic characteristics of patients according to Ki-67

Characteristics	*Ki67<20*	*Ki67≥20*	*p value*
Age, years, median (range)	N: 29 50 (34-68)	N: 77 49 (29-79)	0.90
Menarche age, year, median (range)	N: 26 13 (11-17)	N: 67 13 (11-17)	0.76
Number of births, year, median (range)	N: 26 2 (0-8)	N: 71 2 (0-8)	0.91
Menopause age, year, median (range)	N: 12 50 (42-52)	N: 34 50 (37-56)	0.56
Menopausal status Pre (%) Post (%)	N: 49 12 (24.5) 37 (75.5)	N: 57 17 (29.8) 40 (70.2)	0.66
Body mass index (kg/m^2) Mean ± SD	N: 21 28.1 ± 6.8	N: 59 28.9 ± 4.3	0.53
Hormone replacement therapy	N: 24	N: 71	
Present Absent	3 21	4 67	0.36

N: number of evaluable patients, SD: standard deviation

tients, and 10 of these were in the Ki-67 low group. Twenty patients received neoadjuvant chemotherapy with anthracycline-containing regimen (16 patients were in the Ki-67 high group). Four complete and 14 partial responses were obtained and 2 patients did not respond to neoadjuvant therapy. Three patients who had a tumor less than 1 cm did not receive neoadjuvant or adjuvant chemotherapy. All patients with HR positive disease received hormone therapy. Adjuvant trastuzumab was administered to 67.2% of the patients (16 patients 9 weeks, 51 patients 1 year); 3 patients with less than 1 cm tumor diameter and others who had been admitted before the approval of trastuzumab in Turkey did not receive trastuzumab.

Distant recurrence developed in 23 patients (21.6%). Eight of these patients (34.8%) were in the Ki-67 low group. Recurrence rate was not significantly different in both groups. Recurrence was related with T stage (p=0.02), disease stage (p<0.01), lymphovascular invasion (p=0.02), ER status (p=0.02) and HR status (p<0.01). There was a trend for higher recurrence in patients who did not receive trastuzumab (p=0.08) and in patients with axillary lymph node involvement (p=0.06). Risk factors related to recurrence are presented in Table 3. We did not find any relation between recurrence and other known poor prognostic factors. In multivariate analysis, hormone receptor negativity (p=0.01) and stage (p<0.01) were independent prognostic factors related with recurrence. The most common recurrence site was bone (9 patients, 32.2%). Bone metastasis was more frequent in the Ki-67 low group; however, it was not statistically significant (p=0.09). There was not a significant relation between the Ki-67 level and metastasis site (Table 4). Although without statistical significance, all 3 patients with brain metastasis were in the Ki-67 high group. We were not able to analyze the influence of treatment related factors due to the lack of the number of patients in the Ki-67 low group and the heterogeneity of treatments.

Median disease free survival and overall survival were not reached. During the follow up period, 12 patients (11.3%) died of breast cancer. Death rate was 10.3% (3 patients) in the Ki-67 low group and 11.6% (9 patients) in the Ki-67 high group. Survival rates at the third year for the Ki-67 low group and the Ki-67 high group were 94% and 92%, respectively.

Discussion

Nearly 15-25% of breast cancers are Her-2 positive. Her-2 positivity is a negative prognostic factor. However, Her-2 positive subtype includes a subgroup with good prognosis that can be defined by 70-genes' prognosis signature [12]. However, this method is highly expensive for routine daily use. Hormone receptor positive breast cancers with low proliferation index have a better prognosis than those with high proliferation index. As the proliferative rate that

Table 2. Comparison of tumor pathological characteristics according to Ki-67

Characteristics	*Ki67<20* *N (%)*	*Ki67≥20* *N (%)*	*p value*
Median tumor size, cm (range)	N: 28 3 (0.5-9.5)	N: 76 3 (0.7-10.0)	0.64
Median number of involved lymph node (range)	N: 28 1 (0-19)	N: 76 1 (80-31)	0.73
T stage	N: 29	N: 77	
T1	9 (31.0)	17 (22.1)	
T2	12 (41.4)	41 (53.2)	0.71
T3	6 (20.7)	14 (18.2)	
T4	2 (6.9)	5 (6.5)	
N stage	N: 29	N: 77	
N0	9 (31.0)	27 (35.1)	
N1	10 (34.5)	23 (29.9)	0.72
N2	6 (20.7)	11 (14.3)	
N3	4 (13.8)	16 (20.8)	
Tumor grade	N: 26	N: 72	
G1	2 (7.7)	1 (1.4)	0.14
G2	17 (65.4)	41 (56.9)	
G3	7 (26.9)	30 (41.7)	
Stage	N: 29	N: 77	
Stage 1-2	14 (25.9)	15 (28.8)	0.73
Stage 3	40 (74.1)	37 (71.2)	
Lymphovascular invasion	N: 24	N: 69	
Present	11 (45.8)	48 (69.6)	0.03
Absent	13 (54.2)	21 (30.4)	
Extracapsular involvement	N: 24	N: 63	
Present	5 (20.8)	21 (33.3)	0.25
Absent	19 (79.2)	42 (66.7)	
Perineural invasion	N: 12	N: 40	
Present	6 (50.0)	21 (52.5)	0.87
Absent	6 (50.0)	19 (47.5)	
ER	N: 29	N: 77	
Positive	22 (75.9)	42 (54.5)	0.04
Negative	7 (24.1)	35 (45.5)	
PR	N: 29	N: 77	
Positive	18 (62.1)	33 (42.9)	0.07
Negative	11 (37.9)	44 (57.1)	
Hormone receptor	N: 29	N: 77	
Positive	24 (82.8)	47 (61.0)	0.03
Negative	5 (17.2)	30 (39.0)	

N: number of evaluable patients

is determined by Ki-67 increases, tumor gets negative prognostic features including higher grade, larger tumor size, advanced stage, lymphatic and vascular invasion, hormone receptor negativity and Her-2 positivity [10]. We have proposed that, as in Luminal type breast cancer, Ki-67 may differentiate Her-2 positive tumors with good and poor prognosis. However, there is no agreement on cutoff value for Ki-67 in Her-2 positive breast cancer that will define good and poor prognosis groups.

Patients in both groups had similar demo-

Table 3. Risk factors related to recurrence

Risk factors	*Recurrence* *N (%)*	*No recurrence* *N (%)*	*p value*
Ki-67	N: 23	N: 83	
<20%	8 (34.8)	21 (25.3)	0.36
≥20%	15 (65.2)	62 (74.7)	
T stage	N: 23	N: 83	
T1-T2	13 (56.5)	66 (79.5)	0.02
T3-T4	10 (43.5)	17 (20.5)	
Lymph node involvement	N: 23	N: 83	
Present	19 (82.6)	51 (61.4)	0.06
Absent	4 (17.4)	32 (38.6)	
Stage	N: 23	N: 83	
Stage 1-2	4 (17.4)	50 (60.2)	<0.001
Stage 3	19 (82.6)	33 (39.8)	
Tumor grade	N: 21	N: 77	
G1-G2	12 (57.1)	49 (63.6)	0.58
G3	9 (42.9)	28 (36.4)	
Lymphovascular invasion	N: 20	N: 73	
Present	17 (85.0)	42 (57.5)	0.02
Absent	3 (15.0)	31 (42.5)	
Extracapsular involvement	N: 18	N: 69	
Present	8 (44.4)	18 (30.8)	0.13
Absent	10 (55.6)	51 (69.2)	
Perineural invasion	N: 15	N: 37	
Present	8 (53.3)	19 (51.4)	0.89
Absent	7 (46.7)	18 (48.6)	
ER	N: 23	N: 83	
Positive	9 (39.1)	55 (66.3)	0.02
Negative	14 (60.9)	28 (33.7)	
PR	N: 23	N: 83	
Positive	9 (39.1)	42 (50.6)	0.3
Negative	14 (60.9)	41 (49.4)	
Hormone receptor	N: 23	N: 83	
Positive	10 (43.5)	61 (73.5)	<0.01
Negative	13 (56.5)	22 (26.5)	
Adjuvant trastuzumab	N: 23	N: 83	
No	12 (52.2)	27 (32.5)	0.08
Yes (9 weeks or 1 year)	11 (47.8)	56 (67.5)	

N: number of evaluable patients

graphic features. Tumor characteristics were also similar except lymphovascular invasion and hormone receptor status. It is known that Her-2, ER and PR status are associated with lymphovascular invasion [13]. Lymphovascular invasion was more frequent in the Ki-67 high group. Recurrence was also significantly high in the lymphovascular invasion positive tumors. Frequent presence of lymphovascular invasion in Ki-67 high group may be the sign of aggressive nature of this tumors. This data may indicate that Her-2 positive tumors with lymphovascular invasion may metastasize early. In a previous study, proliferation index was found as an independent predictor of lymph node involvement in invasive breast cancer [14]. However, in this study investigators did not examine Her-2 positivity. In another study, Luminal Her-2 enriched subtype had a high risk for lymph node metastasis, and Luminal A group had the lowest risk [15]. Nodal stage was not affected by Ki-67 status in our positive breast cancer. Ki-67

Table 4. The sites of metastasis according to Ki-67

Metastatic sites	*Ki67<20 N: 8 N (%)*	*Ki67≥20 N: 15 N (%)*	*p value*
Bone			
Yes	5 (62.5)	4 (26.7)	0.09
No	3 (37.5)	11 (73.3)	
Liver			
Yes	5 (62.5)	10 (66.7)	0.84
No	3 (37.5)	5 (33.3)	
Lung			
Yes	2 (25.0)	2 (13.3)	0.48
No	6 (75.0)	13 (86.7)	
Lymph node			
Yes	2 (25.0)	2 (13.3)	0.48
No	6 (75.0)	13 (86.7)	
Brain			
Yes	0 (0.0)	3 (20.0)	0.17
No	8 (100.0)	12 (80.0)	

N: number of evaluable patients

status was not a predictor of lymph node metastasis in the current study.

While Her-2 positive tumors have high Ki-67, HR positive breast tumors have low Ki-67 [10]. In our study 27.4% (28 tumors) of the tumors were in the Ki-67 low group, and ER positivity and HR positivity were more frequent in this group (p=0.04, p=0.03, respectively). Yet, 61% (47 out of 77) of the tumors were also HR positive in the Ki-67 high group, as in Luminal A breast cancers. HR positivity was related with low proliferation in the Her-2 positive tumors. Nevertheless, Ki-67 did not affect recurrence rate and recurrence sites in our study. Recurrence was more frequent in HR negative patients. In multivariate analysis HR status was an independent factor for recurrence (p=0.01). Three-year survival and death rates were similar. Although Ki-67 was related with lymphovascular invasion and HR status, our data indicate that the clinical course of the Her-2 positive breast cancers may be determined by HR status rather than Ki-67. Her-2 positive Luminal type tumors express Luminal type genes including GATA3, BCL2 and ESR1 at a higher rate, whereas Her-2 enriched group expresses tyrosine kinase receptors mostly. These two groups also have different protein expression and somatic mutation profiles [16]. Probably these dissimilar molecular features are the cause of different clinical course.

There are lots of studies evaluating Ki-67 in breast cancer; however in these studies data about Her-2 positive tumors were obtained from subgroup analyses [10,17-19]. To the best of our knowledge, the present study is the first to evaluate the importance of Ki-67 in only Her-2 positive patients in the literature in English language. The main limitation of our study was its retrospective nature. Moreover, median survival time was not reached, but follow up is still ongoing. All patients with HR positive tumors have been treated with hormone therapy; however some of the patients did not receive trastuzumab before approval of this drug in our country. Dual blockage of both ER and HER-2 is an effective treatment and these tumors are also hormone sensitive [20]. In these circumstances ER blockage may have interfere with our result because more than half of the patients in the Ki-67 high group were HR positive. Moreover, we maintain that studies that evaluate the importance of Ki-67 in Her-2 and HR positive breast cancer are needed.

In conclusion, Her-2 positive patients with low Ki-67 and high Ki-67 have similar demographic and pathologic features except lymphovascular invasion and HR status. Her-2 positive breast cancer with good prognosis should not be differentiated with Ki-67. Although hormone receptor positivity was frequent in the Ki-67 low group, disease course had been determined by hormone receptor status in Her-2 positive breast cancer rather than Ki-67 in our patients. Further prospective studies are needed to define the importance of Ki-67 in Her-2 positive breast cancer.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.
2. Revillion F, Bonneterre J, Peyrat JP. ERBB2 oncogene in human breast cancer and its clinical significance. Eur J Cancer 1998;34:791-808.
3. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13-20.
4. Bullwinkel J, Baron-Luhr B, Ludemann A, Wohlenberg C, Gerdes J, Scholzen T. Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. J Cell Physiol 2006;206:624-635.
5. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182:311-322.
6. de Azambuja E, Cardoso F, de Castro G Jr. et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007;96:1504-1513.
7. Perou CM, Sorlie T, Eisen MB et al. Molecular portraits of human breast tumours. Nature 2000;406:747-752.
8. Sorlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-10874.
9. Strehl JD, Wachter DL, Fasching PA, Beckmann MW, Hartmann A. Invasive Breast Cancer: Recognition of Molecular Subtypes. Breast Care (Basel) 2011;6:258-264.
10. Inwald EC, Klinkhammer-Schalke M, Hofstadter F et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat 2013;139:539-552.
11. Edge SB BD, Compton CC, Fritz G, Greene FL, Trotti A. . Breast. AJCC Cancer Staging Handbook (7th Edn). New York: Springer; 2010, pp 417-460.
12. Knauer M, Cardoso F, Wesseling J et al. Identification of a low-risk subgroup of HER-2-positive breast cancer by the 70-gene prognosis signature. Br J Cancer 2010;103:1788-1793.
13. Ugras S, Stempel M, Patil S, Morrow M. Estrogen Receptor, Progesterone Receptor, and HER2 Status Predict Lymphovascular Invasion and Lymph Node Involvement. Ann Surg Oncol 2014;21:3780-3786.
14. Marrelli D, Pinto E, Neri A et al. Mib-1 proliferation index is an independent predictor of lymph node metastasis in invasive breast cancer: a prospective study on 675 patients. Oncol Rep 2006;15:425-429.
15. Howland NK, Driver TD, Sedrak MP et al. Lymph node involvement in immunohistochemistry-based molecular classifications of breast cancer. J Surg Res 2013;185:697-703.
16. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61-70.
17. Cheang MC, Chia SK, Voduc D et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101:736-750.
18. Jerjees DA, Alabdullah M, Green AR et al. Prognostic and biological significance of proliferation and HER2 expression in the luminal class of breast cancer. Breast Cancer Res Treat 2014;145:317-330.
19. Stathopoulos GP, Malamos NA, Markopoulos C et al. The role of Ki-67 in the proliferation and prognosis of breast cancer molecular classification subtypes. Anticancer Drugs 2014;25:950-957.
20. Montemurro F, Di Cosimo S, Arpino G. Human epidermal growth factor receptor 2 (HER2)-positive and hormone receptor-positive breast cancer: new insights into molecular interactions and clinical implications. Ann Oncol 2013;24:2715-2724.