Original Article

Significance of Immunohistochemical Markers in Women with Breast Cancer

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Abstract

Background and Aims: This study aimed to investigate the importance of immunohistochemical (IHC) markers and other prognostic variables in the definition of breast cancer. **Patients and Methods:** Two hundred female patients who underwent breast cancer surgery were classified into two groups according to age: young women (≤45 years; n = 104) and older women (≥65 years; n = 96). Molecular subtypes and local stages were determined. The Kaplan–Meier method was used to estimate the survival curves. The relationships among categorical variables were analyzed using the Chi-square test. **Results:** The difference between the tumor diameter and distribution of Ki-67 levels was significant ($P = 0.001$, $P < 0.05$). T stage, local stage, histological grade, estrogen receptor status, lymphovascular invasion status, axillary nodal state, human epidermal growth factor receptor 2 status, and distribution of molecular subtypes were correlated ($P < 0.05$). The mean disease-free survival rates (DFS) at 1, 2, and 5 years were found 92.9%, 86.5%, and 70.1%, respectively, in the young female group. The DFS rates of older patients were 96.7%, 95.4%, and 84.6%, respectively. **Conclusion:** This study showed that young age was associated with poor prognostic features at the IHC marker level.

Keywords: *Breast cancer, immunohistochemical markers, molecular subtypes*

Introduction

Breast cancer is uncommon in women younger than 40 years.[1]

However, among such individuals, the course is more aggressive than that observed in older individuals. Furthermore, breast cancer is associated with decreased survival and high recurrence rates in younger women. Nevertheless, the biological factors directing this aggressive phenotype need to be elucidated.[2] Studies performed based on genomic profiles in recent years have aimed to define prognostic factors. These results provide convincing evidence that breast cancer has a complex, heterogeneous nature.[3,4] Young age is considered to be an independent variable determining poor survival.[5]

In parallel with the advances made in recent years, immunohistochemical (IHC) markers are now a part of the pathologist's routine.

Some receptor markers, such as estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and Ki-67, are essential for molecular classification.

Specifically, they help to define the tumor phenotype, which can facilitate the determination of the most appropriate treatment strategy. Molecular subtypes also determine disease recurrence, survival, and treatment response. These heterogeneous phenotypic features, which are more pronounced at younger ages, also display aggressive behaviors and contain clues about treatment.[6,7,8]

The aim of the present study was to investigate whether a relationship exists between IHC markers that reflect

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genomic expression in breast cancers observed at a young age at diagnosis and other clinicopathological variables.

These results may help us understand the poor prognosis seen in young women and develop new strategies accordingly.

Materials and Methods

This study was conducted in a tertiary academic hospital after obtaining approval from the hospital's ethics committee (decision number: İ2-118-21).

Informed consent was not obtained from the patients because the study was retrospective.

The study was carried out in accordance with the Helsinki Declaration and followed the Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Two hundred women with breast cancer who were surgically treated (OPERATED) between 2015 and 2020 at our hospital were admitted to the study.

Retrospective data were obtained from medical files and records. Data included patient demographics, surgical procedures, pathological examination (type, size, grade, and lymph vascular and node invasion), estrogen (ER) and progesterone receptor (PR) levels, HER2 status, and KI-67 ratio.

Patients were classified into two groups according to age: young women (≤45 years; n = 104) and older women (≥65 years; n = 96).

Immunohistochemical staining (IHC) was used to establish ER and PR status. HER2 positivity was defined as HER amplification (ratio >2) with 3 + IHC staining or fluorescent *in situ* hybridization (FISH).

The subtype classification was established in accordance with the St. Gallen International Expert Consensus Report (2013).

The primary tumors were classified based on tumor receptors as follows: Luminal A (ER+ and/or PR+ and HER2-, Ki-67 <14%); luminal B/HER2- (ER+ and/or PR+ and HER2-, Ki-67 ≥14%); luminal B/HER2+ (ER+ and/or PR+ and HER2+, any Ki-67), HER2-rich (ER- and PR- and HER2+), and TNBC (ER- and PR- and HER2-).[8]

Another classification of tumors was achieved as luminal or non-luminal based on receptor expression. Finally, tumors were classified as early stage (stages 1 and 2) or locally advanced (stage 3) according to their local stage under the guidance of the American Joint Committee on Cancer (AJCC) staging manual 8th edition.[9]

Data are presented as mean ± standard deviation (SD) in descriptive statistical analyses. Parametric test assumptions were made prior to difference analysis. Shapiro–Wilk test, skewness, and kurtosis were used to control the normality of the data.

If assumptions were made, the difference analysis was performed using the Student's *t* test, and if not, the Mann–Whitney U test was used. Survival curves were estimated using the Kaplan–Meier method. The Chi-squared ($\chi 2$) test was used to analyze the relationship between categorical variables.

Statistical analyses were performed at 95% confidence intervals. Statistical significance was set at $P < 0.05$.

Results

Two hundred female patients were included in the study.

The mean total follow-up time was 54.7 ± 5.5 months. A total of 52% of the patients were under 45 years of age (n = 104), and 48% were over 65 years of age (n = 96). The right breast was affected in 52.5% (n = 105) of patients and the left breast in 47.5% (n = 95). The mean age of the patients

Table 1: Treatment: Surgical

	Young group	Older group	*P*
Surgery *n* (%)	≤45 y/o (*n*=104)	≥65 y/o (*n*=96)	
Mastectomy	51 (25.5)	56 (28)	*P* >0.05
Breast sparing surgery	53 (26.5)	40 (20)	*P* >0.05

y/o, years old

Table 2: Tumor histology and tumor stage

Characteristics *n* (%)	≤45 y/o (*n*=104)	≥65 y/o (*n*=96)	*P*
Tumor histology			
IDC	79 (39.5)	74 (37)	*P* >0.05
ILC	10 (5)	10 (5)	*P* >0.05
Other	15 (7.5)	12 (6)	*P* >0.05
T stage			
T1 (<2 cm)	34 (17)	46 (23)	*P* <0.05
T2 (2-5 cm)	46 (23)	32 (16)	*P* <0.05
T3 (>5 cm)	28 (14)	14 (7)	*P* <0.05
T4	No	no	
Axillary lymphatic metastasis			
Negative	57 (28.5)	86 (43)	*P* <0.05
Positive	39 (19.5)	18 (9)	*P* <0.05
LVI			
Negative	35 (17.5)	52 (26)	*P* <0.05
Positive	69 (34.5)	44 (22)	*P* <0.05
Local stage			*P* <0.05
Early-stage	54 (27)	84 (42)	*P* <0.05
Locally advanced	42 (21)	20 (10)	*P* <0.05

y/o: Years old, IDL: İnvasive ductal carcinoma, ILC: İnvasive lobular carcinoma. LVI: Lymphovascular invasion

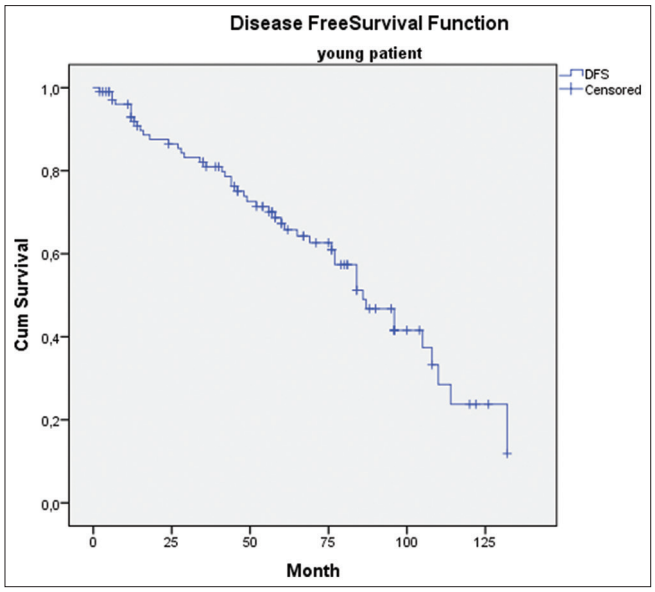

Figure 1: DFS curve of young patients

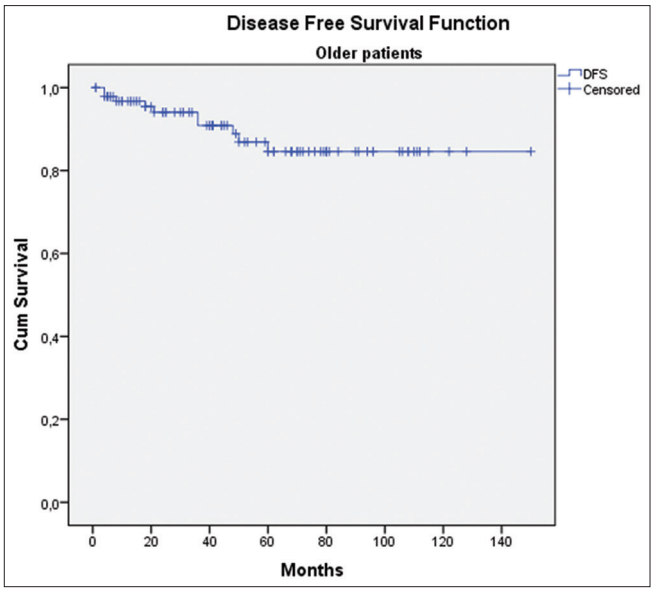

Figure 2: DFS curve of old patients

Table 3: Tumor characteristics: Grade, receptor status, and molecular subtype

Characteristics *n* (%)	≤45 y/o (*n*=104)	≥65 y/o (*n*=96)	*P*
Tumor grade			
Grade 1	7 (3.5)	15 (7.5)	*P*<0.05
Grade 2	30 (15)	50 (25)	*P*<0.05
Grade 3	70 (35)	28 (14)	*P*<0.05
Receptor status			
ER+	41 (20.5)	84 (42)	*P*<0.05
ER−	48 (24)	27 (23.5)	*P*<0.05
PR+	62 (31)	69 (34.5)	*P*<0.05
PR−	42 (21)	27 (13.5)	*P*<0.05
HER2+	55 (27.5)	37 (18.5)	*P*<0.05
HER2−	41 (20.5)	67 (33.5)	*P*<0.05
Molecular subtype			
Luminal A	18 (9)	35 (17.5)	*P*<0.05
Lum B HER−	31 (15.5)	24 (12)	*P*<0.05
Lum B, HER2+	26 (13)	20 (10)	*P*<0.05
HER2+	20 (10)	8 (4)	*P*<0.05
TNBC	12 (6)	6 (3)	*P*<0.05

years; ER+, estrogen receptor positive; ER−, estrogen receptor negative. PR+, progesterone receptor positive; PR−, progesterone receptor negative; HER2+, human epidermal growth factor receptor 2 positive; HER2−, human epidermal growth factor receptor 2 negative; TNBC, triple-negative breast cancer

under the age of 45 was 38.8 ± 5.4 (24–45) years, and the mean age of the patients over 65 years was 71.7 ± 7.6 (65–93) years. The mean tumor diameter (mm) was 44.88 ± 21.54 mm in young patients and 23.77 ± 14.62 mm in older patients. The mean Ki-67 percentages were 44.13 ± 22 in young patients and 25 ± 19.4 in older patients. The mean number of pathological lymph nodes resected was 4.89 ± 3.5 in young patients and 2.42 ± 2 in older patients. Surgical treatment according to the groups is shown in Table 1, the histology and stage of the tumor are shown in Table 2, and the distribution of the tumor according to the grade, receptor status, and molecular subtypes is shown in Table 3.

In the statistical analysis, a significant difference was found in the distribution of tumor diameter and Ki-67 levels between the groups ($P = 0.001$ and $P < 0.05$, respectively). The *P* values of the Chi-squared analysis performed to test the relationship between the qualitative variables are shown in Table 1. The intergroup distribution was associated with T stage, local stage level, ER and HER2 status, histological grade level, LVI status, axillary lymph node invasion status, and the distribution of molecular subtypes ($P < 0.05$). The mean follow-up period of the young patients was 52.7 ± 3 months, and the 1-, 2-, and 5-year disease-free survival rates (DFS) were 92.9%, -86.5%, and -70.1%, respectively. In contrast, the mean follow-up period of older patients was 55 ± 6.5 months, and the 1-, 2-, and 5-year disease-free survival (DFS) rates were 96.7%, -95.4%, and -84.6%, respectively. The DFS curves of young patients are shown in Figure 1, and those of older patients are shown in Figure 2. Intergroup DFS rates were compared using the Log-rank, Breslow, Wilcoxon tests. The difference was significant because the *P* values (0.00,0.003,0.001) were less than 0.05.

Discussion

The aim of this study was to investigate whether a relationship exists between age and hormone receptor status, which was detected by the IHC method and other local prognostic variables.

At the time of diagnosis, young patients were more likely to have larger-sized, ER-negative, HER2B-positive, high Ki-67, and high histological grade tumors with greater axillary involvement and LVI than older patients. Many studies have reported results that are in line with those reported here.[9-12] In their study conducted with genomic variables in 784 breast cancer patients, Anders *et al*. found that young women were associated with lower ER positivity, larger tumors, HER-2 overexpression, lymph node involvement and higher histological grade tumors.[7] They also showed lower quantitative mRNA expression of ER, PR, and HER-2 in tumors seen in young women, which is parallel to the IHC results in the study by,[11] Kollias *et al*.[8] conducted a study with 120 patients under the age of 35, and they divided them into three categories by age. Overall survival (OS) and DFS rates were lower in the younger age group with histologically higher tumors, and LVI was more frequent. No differentiation was observed in terms of tumor size or lymphatic status. The poor prognosis observed in young patients at the time of diagnosis was also explained by the increase in the rate of poorly differentiated tumors at these ages, and it was reported that age alone did not affect them.

Higher HER-2 expression levels have been reported, leading to a more aggressive form of breast tumors at younger ages.[9] However, evidence is insufficient for the significance of this condition in predicting disease-free survival (DFS).[11] It has also been reported that there might be a relationship between young age and increased local recurrence risk.[13] Further, in the present study, the 1-, 2-, and 5-year DFS rates were higher in older women. Specifically, the 5-year DFS rate was 70% for young women and 84.6% for older women. A negative effect on survival in young patients is associated with axillary node involvement and receptor positivity.[2,11] The poor phenotypic characteristics we observed in young women probably affected these results. Keegan *et al*.[12] found that young women had higher HER2+ and TNBC subtype rates than did older women. In addition, young women are at a higher risk than older women for stage III/IV disease and high histological grade tumors at diagnosis. The HER2+ and TNBC subtype rates in the present study were significantly higher in the younger group ($P = 0.021$). In addition, early-stage breast cancer was associated with older age, while young patients were more likely to have locally advanced stage tumors ($P = 0.019$). Carey *et al*.[14] found a high prevalence of TNBC and a low prevalence of luminal A tumors in young patients, which has been found to contribute to poor prognosis.

Kollias *et al.* reported that patients aged < 35 years had high-grade tumors, LVI, and poor prognosis.[8] In line with these findings, in the present study, the presence of LVI ($P = 0.004$) and a high histological grade ($P = 0.008$) were associated with young patients. Young age is now recognized as an independent prognostic factor for the natural course of breast cancer.[2,15,16]

In conclusion, it has been accepted for many years that breast cancer in young women has an aggressive phenotype, but the biological factors that direct this process remain largely unknown.

Ethics committee approval

Approval of the Local Research Ethics Committee of our tertiary hospital was obtained before initiating the study (Ankara University of Medicine Faculty, Cebeci Hospital Turkey, Decision number: İ2-118-21, date: 19-02-2021).

Informed consent

Retrospective study.

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Abbreviations

Immunohistochemical: IHC

Estrogen receptor: ER

Progesterone receptor: PR

Human epidermal growth factor receptor 2: HER2

Lymphovascular invasion: LVI

Fluorescent *in situ* hybridization: FISH

Disease-free survival: DFS

Lum: Luminal

Disease-free survival: DFS

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Conflicts of interest

There are no conflicts of interest.

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