Original Article

Molecular Subtypes of Breast Cancer in Women ≤35 years and >35 years: Does Age Matter?

Abstract

Background: Women diagnosed with breast cancer at young ages (≤35 years) have a substantially shorter overall survival durations. According to molecular pathological classification, triple negative and human epidermal growth factor receptor 2 (HER-2) positive breast cancer subgroups are related with poorer prognosis compared to luminal disease. Based on this rational, the primary objective was to evaluate the impact of age on determining molecular subgroups and whether the different outcomes of patients ≤35 years and >35 years of age are caused by the diversity of molecular subgroups. **Methods**: A total of 216 patients ≤35 years and randomly selected 212 patients of all breast cancer patients >35 years, presented to Ege University Department of Oncology were enrolled in the study. Molecular subtyping was based on estrogen, progesterone receptors (ER, PR), cerb-B2 and Ki-67 proliferation index assessed by immunohistochemistry. Luminal A disease was defined as ER (+), PR (+), cerb-B2 (-), Ki-67 ≤15. Patients with ER/PR (+), cerb-B2 (-)/(+), Ki-67 ≥15 were classified as Luminal B. If all three receptors were negative, it was accepted as triple negative disease and HER-2 positive disease was characterized by lack of hormone receptors and presence of cerb-B2. **Results:** Fifty-two percent in younger group were Luminal B and 19% were triple negative, which composed the largest proportion of the young group. Among the >35 years group the majority was Luminal B (39%) as similar in very young population, however, this was followed by Luminal A (31%) that has favorable prognostic features. Statistically, there was no significant difference in molecular subtypes between the two age groups. However, triple negative subtype and Ki-67 ≥15% which is associated with poorer prognosis, was numerically higher among ≤35 years of group. **Conclusion:** Young women diagnosed with breast cancer have poorer prognosis. However, in our study, there was no statistically significant difference in molecular subtypes between two diverse age groups. This could be explained by small size of the study population, but also could be an indication that age is an independent prognostic factor apart from other clinicopathologic features. Yet, since Luminal B and triple negatives were the largest subgroup in very young population, worse prognosis of the disease in this group may be explained by this diversion.

Keywords: *Breast cancer, molecular subtype, prognostic factor*

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Introduction

The risk of breast cancer increases with age. The majority of cases are diagnosed after the age of 50. The probability of a woman developing breast cancer is 12.8% during her lifetime.[1] The etiology of breast cancer is multifactorial; depends on many factors such as age, gender, genetics, diet, and hormonal disorders. Age and gender are the most important risk factors for breast cancer. Breast cancer occurs 100 times more often in women than men.[2] Advancing age is a risk factor for breast cancer in many women. The development of breast cancer in women aged 30–39 years is 0.04% on average and risk increases each year by 10% and over.[3] Factors associated with the two fold increased risk of breast cancer in women aged 40–49 are to have breast cancer in first-degree relatives and increased breast density (BIRADS 4).[4] Breast cancer is a heterogeneous disease of morphological, molecular and clinical diversity. This heterogeneity could be explained neither by parameters such as tumor size, histological grade, age, nodal involvement nor by biological markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER-2). Molecular classification of breast cancer is illuminated by the development of technologies such as gene expression arrays over the past 10 years. The cellular microenvironment

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and genetic characteristics of the patient affects the pathophysiology and results of disease and response to treatment. Therefore, the treatment of breast cancer must be personalized for each patient. Breast cancer is particularly different in many aspects at a molecular level. These tumor related factors provide information about prognosis and helps to personalized treatment providing the best benefits for each patient. According to molecular pathological classification, triple negative and human epidermal growth factor receptor 2 (HER-2) positive breast cancer subgroups are related with poorer prognosis compared to luminal disease. Women diagnosed with breast cancer at young ages (≤35 years) have a substantially shorter 5-year disease-free survival and overall survival durations.[5] Based on this rational, the primary objective is to evaluate the impact of age on determining molecular subgroups and whether the different outcomes of patients ≤35 years and >35 years of age are caused by the diversity of molecular subgroups.

Methods

The study was planned as a single center, retrospective, descriptive study. A total of 216 patients ≤35 years and randomly selected 212 patients of all breast cancer patients >35 years, presented to Ege University Faculty of Medicine (EUFM) Department of Oncology diagnosed with breast cancer were enrolled in the study. Demographic data, clinicopathological characteristics, and treatment modalities were reviewed and recorded for each patient. Estrogen and progesterone expression were assessed by examining pathology reports of patients. Values of 1% and above considered as "positive," while <1% negative for the percentage of ER and PR expressions.[6] HER-2 expression was evaluated by examining pathology reports of patients. HER-2 expression as assessed in immunohistochemistry (IHC) staining intensity of 0, 1+, 2+ and 3+; 0 and 1+ values are accepted "negative," and 3+ values are accepted "positive." Patients considered as IHC 2+ were categorized according to the FISH test results. FISH-positive patients were classified as "positive," FISH negative ones as '"negative." If FISH test was not performed IHC 2+ patients were classified as "indeterminate."[6] Ki-67 expression was assessed by examining pathology reports of patients. Ki-67 determination technique cannot be standardized because of the addition of a variable factor in the evaluation of these marker (To distinguish luminal B from luminal A tumors cut off point was 13.25%).[7] St Gallen International Expert Consensus adopted unanimously in 2013 that Ki-67 cut-off value 14% is not an appropriate threshold value to define the luminal B subtype. The cut-off value for the majority ranges from 15 to 25%. As a result, a clear consensus has not been achieved on the evaluation of Ki-67 protein. Therefore, as the cut off value of Ki-67 in this study, we agreed to 15%. Ki-67 ≤15% tumors were assessed as luminal A. p53 expression was assessed by examining pathology reports of patients. Values of 1% and above considered as "positive," while <1% negative for the percentage of p53 expressions.

Molecular subtyping was based on estrogen, progesterone receptors (ER, PR), cerb-B2 and Ki-67 proliferation index assessed by IHC. Luminal A disease was defined as ER(+), PR(+), cerb-B2(−), Ki-67≤ %15. Patients with ER/PR(+), cerb-B2(−)/(+), Ki-67≥ %15 were classified as Luminal B. When all three receptors were negative, it was accepted as triple negative disease and HER-2-positive disease was characterized by lack of hormone receptors and presence of cerb-B2.

In the evaluation of the findings obtained in this study SPSS (Statistical Package for Social Sciences) 18 program (SPSS Inc., Chicago, Ill., USA) was used. Data were analyzed using descriptive statistical methods (mean, standard deviation) as well as Chi-square test, Fisher's exact Chi-square test, and Student's *t*-test were used for the comparison of qualitative data. Results were evaluated at 95% confidence interval and P <0.05 was considered statistically significant.

Approval was obtained from the Medical Research Ethics Committee of EUFM Research Ethics Committee with the number 12-6.1/7 dated July 12, 2012.

Results

Pathology reports of 216 women aged 35 years and under presented to EUFM Department of Oncology diagnosed with breast cancer were examined retrospectively. To be able to compare; 276 patients aged 36 and over were randomly selected from the files of 4116 female breast cancer patients. Two hundred and twelve patients whose file containing sufficient information and pathology reports were enrolled in the study. These two groups were compared according to family history, histologic type, tumor size, tumor location, positive lymph nodes, ER, PR, HER-2 expression, Ki-67, p53 status, molecular subtypes, and metastasis situations at diagnosis.

Patient characteristics are listed in Table 1.

The comparison of two groups according to subtype analyses is shown Figure 1. There was no statistically significant difference in molecular subtypes between two age groups. However, triple negative subtype and Ki-67 ≥15% which is associated with poorer prognosis, was numerically higher among ≤35 years of group.

Discussion

Breast cancer comprises 23% of the total cancer cases, causing 14% of the deaths from cancer. Risk of breast cancer increases with age. Significant prognostic factors of breast cancer are; age, tumor size, histological type, positive axillary lymph node, tumor grade, hormone

Table 1: Patient characteristics

	≤35 years, *n* (%)	>35 years, *n* (%)	*P*
Age (*n*=428), median (IQR)	33 (21-39)	50 (43-59)	<0.001
T (*n*=397)			
T1	49 (25.1)	62 (30.7)	0.096
T2	117 (60)	123 (60.9)	
T3-4	29 (14.9)	17 (8.4)	
N (*n*=369)			
N0	63 (32.6)	58 (33)	0.949
N1-3	130 (67.4)	118 (67)	
M (*n*=428)			
M0	196 (90.7)	181 (85.4)	0.087
M1	20 (9.3)	31 (14.6)	
Histology (*n*=428)			
Invasive ductal	147 (68.1)	141 (66.5)	0.100
Invasive lobular	10 (4.6)	18 (8.5)	
Ductal and lobular	19 (8.8)	12 (5.7)	
Inflammatory	18 (8.3)	10 (4.7)	
Other	22 (10.2)	31 (14.6)	
Family history (*n*=350)			
Yes	27 (15.5)	31 (17.6)	0.598
No	147 (84.5)	145 (82.4)	
ER (*n*=428)			
Positive	136 (63)	144 (67.9)	0.281
Negative	80 (37)	68 (32.1)	
PR (*n*=428)			
Positive	129 (59.7)	124 (58.5)	0.796
Negative	87 (40.3)	88 (41.5)	
HER2 (*n*=422)			
Positive	128 (59.8)	123 (59.1)	0.887
Negative	86 (40.2)	85 (40.9)	
p53 (*n*=428)			
Positive	87 (40.3)	94 (44.3)	0.395
Negative	129 (59.7)	118 (55.7)	
Ki-67 (*n*=304)			
Median (IQR)	20 (10-40)	20 (10-30)	0.168
≤14	43 (26.5)	48 (33.8)	0.209
>14	119 (73.5)	94 (66.2)	
Subtype (*n*=320)			
Luminal A	29 (17.5)	48 (31.2)	0.024
Luminal B	87 (52.4)	60 (39.0)	
HER2 enriched	18 (10.8)	18 (11.7)	
Triple negative	32 (19.3)	28 (18.2)	

ER: Estrogen receptor, IQR: Interquartile range, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2

receptor and HER-2 status. Premenopausal young women diagnosed with breast cancer have more aggressive course than postmenopausal aged women.[8] Several studies suggest that young patients have advanced disease at the time of diagnosis.[9] Furthermore, histological grade and lymph node involvement rates are low, hormone receptor expression is high in young patients.[10] Histopathological subgroups of breast cancer can be divided into three groups according to prognostic significance.[11] Mucinous,

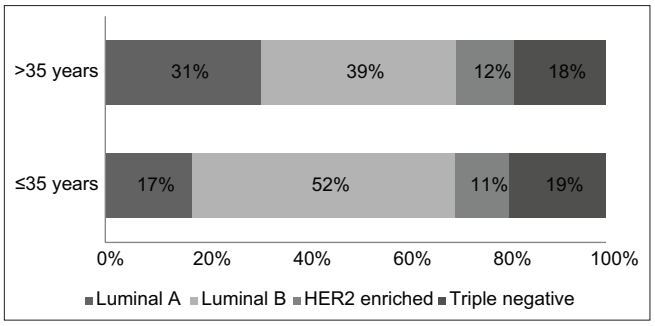

Figure 1: Comparison of molecular subtypes according to different age groups

tubular and papillary have favorable prognosis; medullary and invasive lobulary have intermediate prognosis, invasive ductal and atypical medullary carcinoma have poor prognosis. The most common type of these histopathological subgroups is invasive ductal carcinoma. In addition in our study, invasive ductal carcinoma was the most frequently seen histopathological type.

The presence of lymph node involvement in invasive breast cancer constitutes an important part in staging the disease and provides important prognostic information. Tumors with high grade and nodal involvement are likely to occur at an early age, as around the age of 36 years. Low-grade tumors and tumors without nodal involvement displays a distribution of age with late-onset.[12] However, significant differences between the two groups regarding the number of lymph node involvement was not observed in our study.

Over the past 10 years, many studies describing gene expressions assist in explaining the biology of breast cancer. Gene expression profiling studies have been conducted to understand the genetic heterogeneity of breast cancer; and the combination of clinical parameters with these studies is guiding to improve the classification of these tumors. In addition, gene expressions have clinical significance in predicting prognosis and treatment response and may facilitate the identification of novel molecular targets for drug development. DNA microarray technology that is used for the genetic expression profiles in breast cancer cells, also used to develop breast cancer intrinsic gene set that separates the molecular subtypes with different prognosis and treatment response.[13] Although predictive power of gene expression microarray used for molecular subtyping approach is better than the currently used technique, applicability of these tools in routine clinical use seems remote, because of both the need for greater economic investment and lack of validity and standardization. Despite major advances in biological knowledge, diagnostic methods based on IHC markers are still used in clinical practice.

In our study, in terms of ER, PR, HER-2 positivity, there was no significant difference between the two groups. Colleoni *et al*. have shown that ER, PR negative tumors were more frequent in 35-year-old patients compared to

patients above 35 years of age. Ki-67 expression was also higher in these patients. HER-2 expression was not significantly different. Moreover, the incidence of grade 3 tumors in this study were higher in younger patients.[14] However, in our study; there were no differences in terms of age groups. Only Ki-67 >15%, was higher in the group aged 35 and under. Hormone receptor positivity is an independent prognostic factor in breast cancer. ER and PR positivity indicates response to hormonal therapy and a better prognosis. It has been observed that among invasive tumors, 37%–80% of patients are ER positive and 45%–69% of patients have PR positive disease. The proportion of ER positive cells is associated with differentiation and tumor response to the hormonal treatment. The highest response rate to treatment is in tumors that are both ER and PR positive.[15] Tumors with high levels of ER have relatively better prognoses.[16]

HER-2-neu amplification and overexpression has been reported in 25%–30% of breast cancer patients. In many studies, particularly in patients with positive lymph node, HER-2-neu amplification/overexpression were reported to have a negative effect on disease-free survival and survival durations.[17] According to the study published by Fouratı *et al*.; 4 subtypes defined at the molecular classification and 51% luminal A, 13% luminal B, 13% HER-2+, 22% triple negative were identified in 966 breast cancer patients.[18] In our study, group of 216 patients, 87 (52%) luminal B, 32 (19%) triple negative, 29 (17%) Luminal A, and 18 (11%) HER-2+ were detected. Unlike the common literature, Luminal B was higher in both the age groups. This can be interpreted as a clinicopathologic reflection of the potential geographical and genetic differences. Most of the data related to breast cancer molecular subtypes are from Western sources, this might explain the difference between Western data and our results originating from the Europe-Central Mediterranean region.

Among the molecular subtypes; Luminal B and triple negative tumors were more frequent in ages of 35 and under. The fact that intermediate and poor prognostic features of these two subtypes may be useful in explaining the poor progress of breast cancer at age 35 and under. Carey *et al*. revealed that triple negative breast cancer subtype was more prevalent among premenopausal African-American women with breast cancer whereas the luminal A subtype was less prevalent.[13] Sorlie *et al*. observed basal-like and HER-2+ subtypes associated with the shortest survival times compared to luminal A.[19]

Kronqvist *et al*. documented that the presence of Ki-67 immunopositivity of 10% or above in tumor cells were the most powerful prognostic factor for predicting recurrence and death in early stage breast cancer. In the same paper, patients with <20% of ER positivity and patients with higher than 30% positivity in p53 were associated with an unfavorable outcome of disease.[20]

It is known that 5%–15% of invasive breast cancers have metastases at the time of diagnosis.[21] In our study, metastases were detected in 9.3% of patients under 35 years of age at diagnosis. These results are consistent with literature data. The ratio was 14.6% in 36 years and above. Statistically, there was a significant difference between the two groups.

Ihemelandu *et al*. noted that the average survival time was shorter in patients under 35 years of age, compared to 36 years and over.[22] Although Luminal A subtype is common between the age of 36–50; this group does not have long survival times. The age group of 51–65 years had the longest mean survival time despite having a higher prevalence of basal cell-like, compared with the age groups 36–50 years and 66-–80 years. Accordingly, tumors occurring in the younger and older age groups may be different.

Young women diagnosed with breast cancer have poorer prognosis. New molecular classification and breast cancer prognosis are known to have a strong relationship. However, in our study there was no statistically significant difference in molecular subtypes between two diverse age groups. This could be explained by small size of the study population, but also could be an indication that age is an independent prognostic factor apart from all the other clinicopathologic features. Yet, since Luminal B and triple negatives were the largest subgroup in very young population, the worse prognosis of the disease may be explained by this diversion.

Conclusion

Poor prognosis in younger patients shows that subtypes of breast cancer in these patients have a different tumor biology. Therefore, to plan the right treatment modality, it is important to show the specific prognostic factors in this group. Further studies based on genotypic analyses with larger patient series are required on the subject.

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Conflicts of interest

There are no conflicts of interest.

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