ISSN 2347-6893

Relationship between reproductive risk factors, tumor characteristics and survival in breast cancer molecular groups

Dana Carmen Zaha[1], Felicia Cioara[2], Ramona Suciu[2] , Lucia Daina[2]
[1] Department of Preclinical Disciplines, Faculty of Medicine and Pharmacy, University of Oradea, Romania, danaczaha@gmail.com
[2] Department of Psycho-Neuroscience and Rehabilitation, Faculty of Medicine and Pharmacy, University of Oradea, Romania,
felicia_cioara@yahoo.com, dr_ramona_suciu@yahoo.com, lucidaina@gmail.com

ABSTRACT

Introduction. Breast cancer may be classified into distinct molecular subtypes based on gene expression profiling or immunohistochemical methods. These molecular subtypes are prognostically significant, but their etiologic profiles have not been well established. Our study investigates the relationships between menstrual factors: age of menarche/menopause, irregular/regular menstruation, parity and use of oral contraceptives, tumor characteristics, survival related by the molecular subtypes.

Methods. This study was performed on a group of 173 of patients diagnosticated with breast cancer at The Municipal Clinical Hospital of Timisoara, data being obtained from the medical records of the patients and through a questionnaire sent by post. Molecular classification was made by immunohistochemistry using a panel of four markers: estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 and CK 5/6. All reproductive factors were analyzed in relation to molecular classes and survival.

Results. Younger age at menarche was significantly associated with HER2 and triple-negative subtypes and survival rates were the poorest in women from these groups. The patients with luminal subtype tumors were mostly multiparous whereas the patients with HER2 and triple-negative tumors presented low parity.

Conclusions. From the studied hormonal and menstrual risk factors menopausal status, age at menarche and menopause, oral contraceptives use, parity and family history of breast cancer showed significant trends among tumor subtype and they have a significant impact on molecular breast cancer groups regarding prognosis and survival.

Key words: breast cancer, risk factors, survival.

Academic Discipline And Sub-Disciplines

Human Pathology

SUBJECT CLASSIFICATION

Breast cancer

TYPE (METHOD/APPROACH)

Retrospective observational study

ISSN 2347-6893

INTRODUCTION

Aside from cancers of the skin, invasive breast cancer is the most common carcinoma in women and in the past two decades, the incidence has been increasing. The aetiology of breast cancer is multifactorial and involves diet, reproductive factors (like young age at menarche, older age at first full-term birth, null parity, older age at menopause, oral contraceptives), related hormonal imbalances. More than ninety percent of the new cases and a similar percent of breast cancer deaths occurred in women 40 years of age and older. Men are generally at low risk for developing breast cancer. Breast cancer incidence, as much as most epithelial tumors, increases rapidly with age. During 2004-2008, the median age at the time of the diagnosis was 61 years.[1]

Breast cancer incidence rates are higher in non-Hispanic white women compared to African American women for most age groups. However, African American women have a higher incidence rate before 40 years of age and are more likely to die from breast cancer at every age. Incidence and death rates for breast cancer are lower among women of other racial and ethnic groups than among non-Hispanic white and African American women. In spite of the higher incidence rates, breast cancer death rates are generally lower among non-Hispanic white women compared to African American women.[2]

The prognosis of the disease is benevolent if detected at an early stage. The factors that influence the breast cancer survival are: stage and age at diagnosis, race/ethnicity and socioeconomic factors, obesity, physical activity and diet. Based on the most recent data, relative survival rates for women diagnosed with breast cancer are: 89% at five years after diagnosis, 82% after ten years, 77% after fifteen years.

Epidemiologic studies suggest that some hormone-related breast cancer risk factors may be involve in different molecular types with different prognosis. Breast cancers that are ER+ and/or PR+ are associated with the most favorable prognosis, mainly due to their favorable response to hormonal therapy. Compared to women with ER+ and PR+ tumors, the cases with tumors lacking ER and PR expression have an estimated 1.5- to 2-fold higher risk of death. [3, 4] Breast cancers that overexpress HER2 and triple-negative breast cancers (i.e., ER-, PR-, and HER2-) are also associated with a less favorable prognosis. [5, 6, 7, 8, 9]

The risk factors for breast cancer in women are: age, biopsy-confirmed atypical hyperplasia, genetic mutations for breast cancer, BRCA1 and/or BRCA2, mammographically dense breasts, personal history of breast cancer, high endogenous estrogen or testosterone levels/ oral contraceptive use/ recent and long-term use of menopausal hormone therapy containing estrogen and progestin, high bone density (postmenopausal), high-dose radiation to chest, two/one first-degree relatives with breast cancer, alcohol consumption, early menarche (under 12 years), high socioeconomic status, late age at first full-term pregnancy (after 30 years), late menopause (after 55 years), never having breastfed a child, no full-term pregnancies, obesity (postmenopausal)/adult weight gain, personal history of endometrium, ovary or colon cancer. Some reproductive factors such as age at menarche and menopause, parity, age at first live birth or breast-feeding have in common their effect on the level and duration of exposure to endogenous or exogenous estrogen and could be the risks of different subtypes of breast cancer.

A family history of breast cancer in a first-degree relative is the most widely recognized breast cancer risk factor. Although 20-30% of women with breast cancer have at least one relative with a history of breast cancer, only 5-10% of women with breast cancer have an identifiable hereditary predisposition. BRCA1 and BRCA2 mutations are responsible for 3-8% of all cases of breast cancer and 15-20% of familial cases, but these mutations are present in far less than 1% of the general population. Women with BRCA1 mutations are estimated to have a 44-78% risk of developing breast cancer by 70 years of age. The corresponding risk for BRCA2 mutations is 31-56%.[10, 11]

The events of reproductive life have been considered to be risk factors for breast cancer in women. Breast cancer occurs more frequently among women who have an early menarche, nulliparous or, if parous, have few children with a late age at first delivery. Infertility appears to be a risk factor as may be lack of breastfeeding. Also, late age at menopause increases the risk. Recent data indicate that the age at any delivery, not just the first, is associated with breast cancer risk, with deliveries occurring before the age of 30 having a protective effect. On the other hand, the protective effect of lactation, once considered quite a strong factor, was later given less importance; its impact appears limited to longterm cumulative breast feeding, preferably exceeding two years. Other important risk factors are exogenous hormones. Two major types of hormonal compounds have been evaluated in relation to breast cancer: oral contraceptives and menopausal replacement therapy. The evidence suggests a small increase in the relative risk associated with the use of combined oral contraceptives, especially among current and recent users, which is not related to duration of the use and type or dose of preparation and may be partly linked to detection bias.

The estrogen excess hypothesis is central, stipulating that breast cancer risk depends directly on breast tissue exposure to estrogens. In vitro studies show increased breast cell proliferation and inhibition of apoptosis. Animal studies show increased rates of tumor development when estrogens are administered. A second major theory, the estrogen plus progesterone hypothesis, postulates that compared to exposure to estrogens alone (as in postmenopausal women not using exogenous hormones), risk of breast cancer is further increased in women who have elevated plasma and tissue levels of estrogens in combination with progestogens. This theory is supported by observations that proliferation of mammary epithelial cells is being increased during the luteal phase of the menstrual cycle, compared to the follicular phase. Among premenopausal women, several studies have not shown any clear association between breast cancer risk and circulating levels of estrogens or progesterone. Despite the large body evidence regarding effects of hormonal birth control and hormone replacement therapy on breast cancer, it is still unclear whether women are more susceptible to the effects of these exogenous sex steroids at specific points during the life course. Our study provided an opportunity to answer a part of these questions in relation with molecular subgroups.

ISSN 2347-6893

MATERIAL AND METHODS

This study was performed on a sample of patients diagnosticated with breast cancer at The Timisoara Municipal Clinical Hospital, between 2000-2005, data being obtained from the medical records of the Department of Pathological Anatomy and was completed with clinical and therapeutic data from the Oncological Surgery, Radiotherapy and Chemotherapy Departments. Other information was obtained through a questionnaire sent by post. The 173 selected cases were completely classically morphologically evaluated. Data included: grade, lymph node involvement; estrogen receptor (ER), progesterone receptor (PR), HER2, CK 5/6 status were all imunohistochemicaly determined. Based on these the breast tumors have been categorized in the four molecular classes: triple-negative, luminal A, B, HER2. Detailed information regarding the use of oral contraceptives or menopausal hormone therapy, the number of births and pregnancies were collected by means of postal questionnaire or medical records. Additionally, around 30% of cases were contacted by telephone to complete missing or ambiguous responses. Data regarding the deaths have been obtained from medical records, the deaths archives of the Timisoara City Hall and postal questionnaire. Age at menarche was classified as not older than 11, between11-14 and older than 14 years. Menopause was defined as the age of the last menstrual period or bilateral oophorectomy. Age of menopause was grouped as younger than 50 years, 50 to 55 years and as an older than 55 years. Irregular menstruation was either absent or present during the lifetime. Regarding parity, the cases were classified as follows: nulliparous, uniparous and multiparous.

The relationships between molecular classification and risk factors of interest were evaluated using chi- square tests of association and effect size (φ Cramer). All probability values of p less than 0.05 were considered significant. According to Cohen the φ Cramer value determines:

- 0.10 – low effect

- 0.25 – medium effect

- 0.40 – high effect

Breast cancer mortality rates were calculated by hormonal and menstrual factors according to the number of breast cancer deaths in the interest groups.

Table 1 Distribution of reproductive factors in relation to molecular subgroups

Parameter	Total (n=173)	Luminal A (n=92)	Luminal B (n=32)	HER-2 (n=5)	Triple-negative (n=44)	P value*
Age of patiens	31-85	31- 85	36-83	37-68	34-78	
Mean age	56	58	54	52	53	
Irregular menstruation						
No	147	76	28	5	38	
Yes	26	16	4	0	6	0.678
Parity						
Nulliparous	24	10	2	3	9	
Uniparous	53	28	10	0	15	
Multiparous	96	54	20	2	20	0.02
Age at menarche						
Under 11 years	56	21	7	3	25	
Between 11- 14 ys	30	19	5	1	5	
Past 14 years	87	52	20	1	14	0.0024
Age at menopause						
Under 50 years	24	5	4	3	12	
Between 50- 55 ys	25	10	7	1	7	
Past 55 years	124	77	21	1	25	0.0076
Oral contraceptives use						
No	149	73	29	3	44	
Yes	24	19	3	2	0	0.002
History of breast cancer						
No	167	89	31	5	42	

ISSN 2347-6893

Yes	6	3	1	0	2	0.94
Family history of breast cancer(first degree relatives)						
No	138	78	28	3	29	
Yes	35	14	4	2	15	0.028

*Pearson chi-square tests of association between classes

RESULTS

All reproductive factors were analyzed in relation to molecular classes and survival. Menopausal status (p=0.00), age at menarche (p=0.0028), age at menopause (p=0.0076), oral contraceptives use (p=0.002), family history of breast cancer (p=0.028) and parity (p=0.02) showed significant trends among tumor subtype (table 1). Table 2 shows the distribution of tumor characteristics among the four molecular subtypes and five-year relative survival rates described next.

Table 2 Tumor characteristics for breast cancer subtypes and relative survival rates

Parameter	Luminal A (n=92)	Luminal B (n=32)	HER 2 (n=5)	Triple-negative (n=44)
Clinical stage at diagnosis				
stage I	6	1	0	1
stage II a	63	16	2	25
stage III a	21	13	3	18
stage IV	2	2	0	0
Tumoral size (cm)				
Mean	2,5	3,1	4,4	4,5
Under 2.5 cm	48	8	0	3
Between 2.5 and 5 cm	36	13	1	21
More than 5 cm	8	11	4	14
Grading				
Low	9	2	0	1
Medium	81	26	2	20
High	2	4	3	23
Lymph node involvement				
Absent	38	11	2	12
Present	54	21	3	32
Histology				
Ductal	58	19	5	28
Lobular	7	1	0	0
Mixt (ductal and lobular)	18	8	0	5
All other	9	4	0	11

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Number of deaths at the end of five year follow-up	13	9	2	19
Five-year relative survival rates	85.8%	72%	60%	56.8%

Luminal A cancers are the highest proportion (53.1%); they tend to have the most favorable long-term survival, as the overall 5-year relative survival has been 85.8%. The luminal subtypes are low/medium grade, have a high expression of ER and PR and HER2 negative; including the highest proportion of stage I - II and well/ moderately-differentiated lesions. Ages of the patients ranged from 31 to 85 years with a mean age of 58 years. Amongst this group, the majority presented regular menstruation, was postmenopausal, did not use hormonal contraceptives and presented a low percentage of personal or familial medical history to first degree relatives with cancer. As to the number of pregnancies, a low percentage (10.8%) was nulliparous, the majority being multiparous (58.6%). 56.5 percent of the patients experienced menarche at an age older than 14, a percentage of 83.6% representing the age older than 55 at which menopause occurred.

Luminal B (18.4 %) cancers have a lower expression of ER and PR, with HER2 positive or negative. Luminal B cases have shown poorly differentiated cancers, in a larger percentage than luminal A cases, this probably being one of the explanations for the difference between the two kinds of survival rates. In addition, patients with luminal B subtype carcinomas had an increased tendency to involve lymph nodes compared to patients with luminal A subtype tumor. Patients with luminal B subtype tumors were between the same age limits, but younger than those with luminal A subtype, the mean age being 54. The distribution of menstrual and hormonal factors was similar to those from luminal A type: the patients were mostly multiparous, with regular menstruations, and the use of contraceptives was reduced. The appearance of menarche was, for 62.5% of the patients, at an age older than 14, whereas menopause occurred after 55 for 65.6% of the patients. In regards to the personal medical history of breast cancer, this was not met at all, and the familial medical history of first degree relatives having breast cancer was present in only 12.5% of the cases.

Most women with BRCA1 mutations generally develop triple-negative breast cancer and a relatively high percentage (25.4%) of tumors was this type. In this study, BRCA mutations were not determined, but 34.2% of the cases had first-degree relatives with history of breast cancer. Most cases were invasive ductal carcinomas and two third presented nodal metastasis at the time of diagnosis, but nine cases were medullar types. Patients' age was between 34 and 78, with an average of 53 and it is presented largely in premenopausal women. The majority presented regular menstruations and did not use hormonal contraceptives, these being, in fact, characteristic to the whole studied group. Minimum inequalities concerning the proportion between null-, uni-, and multiparous, have been noticed but the highest percentage belongs to the low parity patients. However, significant is the occurrence of menarche at less than 14 years for 68% of the patients in correlation with the appearance of menopause at fewer than 55 years in proportion of 43.1%. Our data sets have revealed that the triple-negative subtype has a poor prognosis, the average survival rate in 5 years being 56.8%.

ISSN 2347-6893

Table 3 Chi-square test and effect size for survival evaluation

Factors	All cases		Survival				p-value	φ Cramer value	Interpretation (Effect size)
			Yes		No				
	N	%	N	%	N	%			
Irregular menstruation							0.402	0.064	Very small
No	147	84.9	112	86.2	35	80.9			
Yes	26	15	18	13.7	8	19.1			
Parity							0.00000002	0.450	High
Nulliparous	24	13.8	7	5.3	17	39.5			
Uniparous	53	30.6	39	30	14	32.5			
Multiparous	96	55.4	84	64.6	12	27.9			
Age of menarche							0.00003	0.346	Medium
Under 11 years old	56	32.3	32	24.6	24	55.8			
Between 11-14 ys	30	17.3	20	15.3	10	23.2			
Past 14 years old	87	50.3	78	60	9	20.9			
Age of menopause							0.173	0.181	Small to medium
Under 50 years old	24	22.4	17	19.3	7	36.8			
Between 50-55 ys	25	23.3	20	22.7	5	26.3			
Past 55 years old	124	54.2	51	57.9	7	38.8			
Menopausal status							0.006	0.209	Medium
Premenopausal	66	38.1	42	32.3	24	55.8			
Postmenopausal	107	61.8	88	67.6	19	44.1			
Oral contraceptives use							0.983	0.002	Very small
No	149	81.5	106	81.5	35	81.3			
Yes	24	18.5	24	19.5	8	18.6			
History of BC							0.001	0.256	Medium
No	167	97.1	129	99.2	38	88.3			
Yes	6	2.8	1	0.7	5	11.6			
Family history of BC (first degree relatives)							0.00004	0.343	Medium to high
No	138	79.7	114	87.6	24	55.8			
Yes	35	20.2	16	12.3	19	44.1			

HER2 cancers have high levels of HER2 expression, with minimal expression of ER and PR. The HER2 subtype (2.8 %) is more likely to be high grade and poorly differentiated and was more likely to involve axillary lymph nodes. All five tumors of HER2 subtype were histological invasive ducal carcinomas and had the poorest prognosis, the overall 5-year relative survival being 60%. The age of patients ranged from 37 to 68 years with a mean age of 52 years, 4 out of 5 were premenopausal, with the occurrence of menopause at less than 50 years for 3 out of 5 patients and they experienced menarche before 14 years. All the patients of this group had regular menstruations and 3 out of 5 of them were nulliparous. The patients did not present personal clinical record of breast cancer; instead, 2 out of 3 had first degree relatives with this disease.

ISSN 2347-6893

Considering the data regarding the overall survival depending on reproductive risk factors, using the Chi- square test to analyze the existence of a relationship between survival and independent variables (reproductive factors) that can influence it, significant statistical differences regarding parity, age at menarche and age at menopause have been noticed (table 3). On the other hand, the value of Chi-square shows if the two variables are related or not while the value of φ Cramer (effect size) shows the intensity of the relationship between the variables (dependence – independence). We observed that survival is significantly influenced by parity, age at menarche, menopausal status, family and personal history of breast cancer.

DISCUSSION

It has been hypothesized that the risk of different molecular types of breast cancer is associated with aspects of reproductive and menstrual history. Risk factors in breast cancer do not necessarily have the same effect in young and older patients.

Age is the most significant risk factor for breast cancer, with breast cancer being rare in women younger than 25 years, incidence increasing with age. Even though statistic significant differences in regards to age have not been observed, the mean age of our patients was 56, majorly lower than the data in scientific literature, where the mean age at breast cancer diagnosis is 61 years of age; the youngest patients presented triple-negative tumors and HER2 higher grade and larger tumors, and the oldest ones had luminal tumors according to other studies.[1,12,13]

Many studies have calculated that approximately one third of breast cancer cases are associated to late age at first birth or nulliparity. The finding of a protective effect of pregnancies supports the evidence suggesting that pregnancies protect against breast cancer through a hormonal mechanism. It has been reported nulliparity was associated with decreased risk of triple-negative breast cancer and an inverse association with parity has been established for ER+ diseases.[12, 14, 15] On the other hand, multiparity was associated with an increased risk of ER- PR- cancer, but this risk was reduced by breastfeeding, such that multiparous women with a history of breastfeeding were no longer at increased risk.16 In our study, multiparity occurred in luminal tumors while low parity (nulliparous and uniparous) was in triple negative tumors and HER2 type in almost the same proportion with multiparity. Because of the small number in HER2 tumors further exploration of breast cancer risk associated with low parity is needed.

Early age at menarche was associated with a reduced risk of luminal A tumors, but in women younger than 40, earlier age at menarche was associated with an increased risk of breast cancer in both ER+PR+ and ER−PR− tumors and For ER+PR+ tumors, women with an age at menarche of age 14 or older were 50% less likely to be diagnosed with breast cancer than those with an age of menarche of under 12.[12,17] In our study the majority of the cases experienced menarche after 14 years old for luminal cases where the number of deaths is relatively low. Triple-negative and HER2 tumors were related to the earliest age at menarche, thus confirming Xiaohong R. Yang et al.'s results. [18]

In regards to the menopausal status, the triple-negative and HER2 tumors were especially premenopausal, and the luminal ones predominated over the postmenopausal. Menopause settled in before 55 years old in a part of the luminal B, HER2, and triple-negative tumors.

There is a significant heterogenity concerning the association of oral contraceptive (OC) use and breast cancer risk between triple negative breast cancer and luminal among young women. Some studies have shown that OC use increases a young woman's risk of breast cancer; although others suggest that the risk may be limited to recent use; oral contraceptive use more than five years, compared with never use, was positively associated with triple-negative nulliparous women. [16]

There is evidence about a higher risk among women with a family history of breast cancer, while others have found little or no such evidence. Oral contraceptives use was not associated with either subtype.[19, 21] Among women under 40 years, the relative risk of triple negative tumors associated with OC use more than a year was 4.2, whereas there was no significantly increased risk with OC use for luminal tumors among women [20 The objective of this study was to determine what particular aspects of OC use could be an important prognostic factor for a certain type of breast cancer. Low percentages (18.4%) from the cases have used oral contraceptives for more than five years and belong to the HER2 subtype.

Personal breast cancer antecedents were met only at four patients with luminal, and two respectively triple-negative tumors. A number of 35 patients had a history of breast cancer in their families, in a first-degree relative, having had triple-negative and HER2 tumors in proportions nearly equal. As we can see in the table 3, survival curves showed significant differences in survival regarding age at menarche, parity, menopausal status, and family and personal history of breast cancer.

CONCLUSION

Analysis of data from this work showed differences in risk of breast cancer factors by tumor subtypes, supporting the view that the molecular classification might be relevant for tumor etiology and we previously confirmed reported differences in tumor pathology and patient characteristics by breast cancer subtypes. [6, 7, 8, 9]

We found that luminal breast cancers were numerically predominant and were different in pathologic characteristics from non-luminal tumors. Luminal A tumors included the highest frequency of small tumors, the lowest frequency of poorly differentiated carcinomas and the best survival rates. In contrast, HER2-expressing and triple-negative tumors showed the

highest frequency of poorly differentiated carcinomas associated with poor survival and analyses indicating that HER2 gene amplification, which generally corresponds to strong immunohistochemical expression, is a poor prognostic factor.

Some studies have found younger ages at diagnosis for triple-negative tumors and associations with BRCA1/2 mutations.[7, 20] Our results are in agreement with previous demonstrations which triple-negative tumors were significantly associated with early ages at menarche. [18, 22]

Demographic factors demonstrated that patients with luminal breast disease were more likely to be older and diagnosed at an earlier stage, while patients with triple negative tumors were more likely to be younger. Both late age at menopause, age at menarche after 11 years old and multiparity were found to be associated with luminal disease.

Although based on small numbers, our analysis suggests that HER2-expressing tumors may have different risk factor associations compared to luminal A tumor. Early age at menarche, family history of breast cancer (first degree relatives) and premenopausal status was associated with the risk for HER2 and triple-negative cancers.

A part of these risk factors have in common their effect on the level and duration of exposure to endogenous or exogenous estrogen. Although many of these factors have been shown to contribute to elevated systemic levels of estrogens, relationship between high serum levels and development of hormone receptor positive tumors has not been established.

However, there are several potential limitations to the study design that could affect the interpretation of the results. Limitations of this study include statistical power limited by the number of cases, too small for HER2 and triple-negative in relationship with breast cancer risk factors. Future studies with an increased number of cases further can define better the etiology of breast cancer subtypes. We used IHC to classified tumors and we applied a semi quantitative scoring system. However, the evaluation of TMA blocks may lead to misclassification of marker expression for tumors with regional differences in marker expression levels, which would tend to dilute associations with exposures.

In conclusion, our results support the hypothesis that breast cancer risk factors vary by molecular breast cancer subtypes at least regarding parity, age at menarche and menopause, menopausal status and personal history of breast cancer.

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