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Prognostic factors and molecular subtypes in young women with breast cancer

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ABSTRACT

Introduction: Because of its high incidence, breast cancer is the subject of numerous studies today. Despite being an uncommon disease in young women, when it affects this population, it tends to be more aggressive and has high mortality rates. **Objective:** The objective of this study was to evaluate the prognostic factors present in the immunohistochemical profile of young women with breast cancer, comparing the age groups of very young women (<35 years old — Group I) and young women (between 35 and 40 years old — Group II), to see if the data obtained match what is reported in the literature. **Methods:** A cross-sectional study was carried out, analyzing the immunohistochemical tests of 90 female patients with invasive breast carcinoma. The groups were classified on the basis of molecular subtype: luminal A, luminal B, hybrid luminal, human epidermal growth factor receptor 2 positive and triple-negative. **Results:** The histological type with the highest incidence was invasive breast carcinoma of no special type. The most frequent molecular subtypes were luminal B and triple-negative. With regard to estrogen and progesterone receptors, there was a slight predominance of positive receptors. Ki-67 levels showed that in the triple-negative and human epidermal growth factor receptor 2 positive subtypes, there was a predominance of high cell proliferation index. **Conclusion:** In the population of young women in this cohort of patients, there was agreement with literature data regarding the predominance of the invasive carcinoma of no special type histological type and the luminal B and triple-negative molecular subtypes, and the presence of high cell proliferation rates, attesting to the higher prevalence of more aggressive tumors in the younger population. There was also no statistically significant difference in all aspects analyzed when comparing Groups I and II. However, a higher frequency of negative hormone receptors or overexpressed human epidermal growth factor receptor 2 molecular subtypes was not detected, characteristics that are common to young women with breast cancer, which has been pointed out in several studies worldwide.

KEYWORDS: breast cancer; immunohistochemistry; prognosis; biomarker.

INTRODUCTION

It is a well-documented fact that breast cancer is the malignant neoplasm with the highest incidence in the female population worldwide, excluding only non-melanoma types of skin cancer[1]. Despite being relatively uncommon in young women, breast cancer is the leading cause of death from malignant neoplasms in women under 45 years of age[2]. Data presented by the World Health Organization (WHO), referring to a population of 100,000 women evaluated in 2020, showed the following results: incidence of 58.5% for all ages and 10.3% for under 40 years[3]; and percentage of deaths of 17.7% for all ages and 1.8% for under 40[3].

According to the Brazil's National Cancer Institute (INCA), the estimate of breast cancer cases in Brazil for each year of the 2020-2022 triennium is 66,280[4]. Regarding mortality, 18,068 deaths were recorded in 2019, of which 1,246 were women under the age of 40[5]. Such incidence and mortality values demonstrate the need for extensive research on the subject, focusing on early diagnosis through screening programs and determination of its main prognostic factors.

Numerous studies indicate that the age group with the highest incidence of breast cancer is between 50 and 65 years old, which is nine times greater than in women under 40[6], making this cancer an event of low incidence in younger women[7].

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Regarding the number of cases of the disease, 10% were in the 34-to-44-year age group, while 2% between 20 and 34 and 0.1% under 20[7]. That is, the younger the patient, the lower the chances of developing breast cancer. On the other hand, while the most prevalent age group (50–65) has tumors with a better prognosis and easier diagnosis, young women have the worst prognosis and significantly lower survival[8]. Although there is no consensus, a young female patient it is classified as being under 40 years[9].

According to the Brazilian Society of Mastology, breast cancer screening should be done through an annual mammogram for women over 40 years old. The exclusion of young women from mass screening, justified by the low incidence of the disease, can delay early diagnosis. In this age group, cancers of the human epidermal growth factor receptor 2 (HER2) and triple-negative subtypes are common, which are usually detected clinically, precisely when they have already reached large dimensions. Therefore, it is assumed that the higher rates of invasive tumors in relation to ***in situ*** tumors, in the population under 40 years old, must be attributed to the fact that many cases are diagnosed at an advanced stage[6].

The non-inclusion of young women in screening programs and the more aggressive tumor biology not only lead to delayed diagnosis, increasing mortality rates, but also determine that epidemiological studies of breast cancer are mostly composed of older women, underestimating thus the values referring to the young women, considering them not very representative[9].

However, it is known that mammographic screening in young women loses part of its sensitivity and specificity because of high breast density. This can also lead to unnecessary radiation exposure, to high rates of false positives or a false sense of security. The ideal would be individualized screening programs, taking into account the risk factors of each patient, such as family history and genetic mutations[10].

Of all the genetic mutations associated with breast cancer, those of the BRCA1 and BRCA2 genes are important negative prognostic factors[9], more commonly found in young women, reaching 40% in familial breast cancers[11]. These mutations have a prevalence of 10% in women younger under 40 years old and 30% in those under 30[7] and are associated with the development of basal-like tumors (negative for ER, RP and HER2 and positive for CK5), characteristic of the younger population[12]. In light of this, it can be inferred that although the development of breast cancer in very young women (<35 years) is a rare event, when it occurs, the chances of involvement of the BRCA genes are greater, and consequently, the greater is the probability of more aggressive molecular subtypes developing.

Thus, women with a BRCA mutation (1 or 2) are considered high-risk patients and fall into another screening profile, where mammography interspersed with magnetic resonance imaging is recommended every six months, starting at age 30[13].

Since breast cancer is a disease with heterogeneous characteristics, several studies approach the oncological profile of patients through the analysis of prognostic factors and molecular biology, so the stratification of tumors into different degrees of aggressiveness and risk of recurrence makes it possible to identify the behavior of the cancer and individualized treatments.

Immunohistochemistry is routinely used in clinical practice because of its lower cost and better accessibility for classifying molecular subtypes. The accuracy of this methodology has already been demonstrated as safe in previous studies, detecting 85% of agreement between the immunohistochemical and molecular subtypes[14]. However, comparing the molecular classifications determined by immunohistochemistry and by the microarray PAM50 test (molecular assay of non-routine use, due to its low cost-benefit), important discrepancies were found[15].

Characteristics found in pathological and immunohistochemical tests, such as a higher frequency of high histological and nuclear grade, positive angiolymphatic invasion, negative hormone receptors, high cell proliferation index (CPI) and higher incidence of triple-negative molecular subtypes and amplified HER2, contribute to a worse prognosis in young women[13,16,17]. This fact confirms what was previously inferred, verifying that the tumors found in young women tend to be more aggressive.

On the basis of the information presented, this study was developed with the objective of analyzing the molecular profiles of women under 40 years of age, according to immunohistochemistry, and comparing them with the data contained in the literature.

This study was approved by the Research Ethics Committee of the University of Taubaté (protocol CAAE-42804120.1.0000.5501) according to Resolution CNS/MS No. 466/12.

METHODS

A cross-sectional study was carried out with the evaluation of prognostic factors, obtained through the analysis of immunohistochemical tests, of 90 women between 21 and 39 years old, from 2015 to 2020. The reports were provided by a pathological anatomy laboratory in the city of Taubaté (SP). Tumors were evaluated according to estrogen and progesterone hormone receptors, CPI (Ki-67) and HER2 expression. Cases with indeterminate HER2 not submitted to FISH (fluorescence ***in situ*** hybridization) analysis were not included. Examinations with incomplete immunohistochemistry data were excluded.

The classification according to the immunohistochemical profile is based on the evaluation of estrogen and progesterone receptors, CPI (Ki-67, referring to a nuclear protein strictly related to cell proliferation) and the biomarker HER2[18].

Tumors were classified into five subtypes: luminal A, luminal B, hybrid luminal, HER2 and triple-negative. This classification was performed according to the Table 1 below:

For Ki-67, a cutoff point of 14% was used for the differentiation of cancers into luminal A and luminal B, based on the criteria established by Cheang et al.[18].

The histological type, determined according to the WHO classification, was obtained by anatomopathological examination, including invasive carcinoma of no special type (NST) and special carcinomas. Reports with a diagnosis of ductal carcinoma *in situ* and lobular carcinoma *in situ* or with another diagnosis of non-carcinoma malignant breast cancer were excluded.

Patients were divided into two subgroups: less than 35 years old (Group I) and from 35 to 39 years old (Group II), to compare the prognostic factors found in different age groups, as was done in other studies[1].

To compare young and very young women, the G (Williams) and $\chi 2$ tests were performed, where $p<0.05$ was considered significant. The database was analyzed using the BioEstat 5.3 program.

In evaluating cell proliferation rates in triple-negative and HER2 tumors, we obtained the following results: triple-negative, 4 (16.67%) with low CPI, 2 (8.33%) with moderate CPI and 18 (75%) with high CPI; in HER2 tumors, 3 cases (27.27%) with low CPI, 2 (18.18%) with moderate CPI and 6 (54.54%) with high CPI.

The pathologists classified the tumors according to the CPI and defined it as low, moderate and high, according to the Ki-67 values, that is, low (<15%), moderate (from ≥15% to ≤20%) and high (>20%). According to the manual for standardization of histopathological reports[19], the Ki-67 value above 15 to 20% is considered high; however, the literature does not establish a specific cut-off point, recommending only that the percentage of stained nuclei be mentioned in the histopathological report[19].

The comparative analyses of the two groups are described in the following Table 2 and there were no statistical differences in the parameters analyzed between the two groups:

RESULTS

The number of patients included in the study, diagnosed with breast carcinoma, was 90, of which 33 were between 23 and 34 years (Group I) and 57 were aged 35 to 39 years (Group II).

Evaluating the histological types, the most prevalent was non-special invasive carcinoma, present in 85 women (94.44%), and five special subtypes: invasive metaplastic, invasive metaplastic with myogenic and rhabdomyoplastic differentiation, invasive cystic adenoid, invasive colloid and invasive lobular.

Regarding the 90 patients, the most prevalent molecular subtype was luminal B, present in 26 women (28.89%), and non-basal triple-negative, in 24 (26.67%), followed by luminal A, detected in 19 (21.11%), HER2 in 11 (12.22%) and hybrid luminal in 10 (11.11%). As for hormone receptors, 53 ER+ (58.89%), 37 ER- (41.11%), 49 PR+ (54.44%) and 41 PR- (45.56%) were found.

Table 1. Classification of the molecular subtypes according to immunohistochemical profile[13].

Molecular subtype	Immunohistochemical profile
Luminal A	ER+ and/or PR+, HER2- and Ki-67<14%
Luminal B	ER+ and/or PR+, HER2- and Ki-67≥14%
Hybrid luminal	ER+ and/or PR+, HER2+ and any Ki-67
HER2	ER-, PR- and HER2+
Non-basal triple-negative	ER-, PR- and HER2-

HER2+: Human Epidermal growth factor Receptor-type 2 positive; HER2-: Human Epidermal growth factor Receptor-type 2 negative; ER+: Estrogen receptor positive; ER-: Estrogen receptor negative; PR+: Progesterone receptor positive; PR-: Progesterone receptor negative.

Table 2. Results obtained in the sample and respective p-values.

Parameters		<35 years n (%)	35–39 years n (%)	p-value
Molecular subtype	Luminal A	6 (18.18)	13 (22.80)	0.9257 *
	Luminal B	11 (33.33)	15 (26.32)	
	Hybrid luminal	3 (9.09)	7 (12.28)	
	HER2+	5 (15.15)	6 (10.53)	
	Non-basal triple-negative	8 (24.24)	16 (28.07)	
Estrogen receptor	ER+	19 (57.58)	34 (59.65)	0.9764 **
	ER-	14 (42.42)	23 (40.35)	
Progesterone receptor	PR+	18 (54.55)	31 (54.38)	0.8376 **
	PR-	15 (45.45)	26 (45.62)	
HER2	HER2+	8 (24.24)	13 (22.80)	0.9176 **
	HER2-	25 (75.76)	44 (77.19)	
Non-basal triple-negative tumor (CPI)	Low	1 (12.50)	3 (18.75)	0.6250*
	Moderate	0	2 (12.50)	
	High	7 (87.50)	11 (68.75)	
HER2 tumor (CPI)	Low	1 (20.00)	2 (33.33)	0.9038*
	Moderate	1 (20.00)	1 (16.67)	
	High	3 (60.00)	3 (50.00)	

HER2+: Human Epidermal growth factor Receptor-type 2 positive; HER2-: Human Epidermal growth factor Receptor-type 2 negative; ER+: Estrogen receptor positive; ER-: Estrogen receptor negative; PR+: Progesterone receptor positive; PR-: Progesterone receptor negative; * G test (Williams); ** χ^2 test.

DISCUSSION

Regarding the histological type, the results obtained in this study showed that invasive ductal carcinoma was the most common (94.18%). Similar data were found in a national study that evaluated 12,689 young women[1], demonstrating a frequency of 90.7% of invasive ductal carcinoma, with no statistical difference between two age groups: younger than 35 years and 35 to 39 years.

The study conducted in the United Kingdom[20], published in 2013, analyzed about 3,000 women under 40 years old, finding similar percentages as in the present study: 86.5% were diagnosed with invasive ductal carcinoma, 4.5% with lobular carcinoma and 0.4% with metaplastic. Regarding hormone receptors, the same study[20] found 65.9% ER+, while our study here found 58.89% ER+ in the total number of women analyzed. Another similarity of the studies was the proportion of HER2+ tumors, so that by adding the cases with hybrid luminal and those with overexpressed HER2, 24.3% were found in the British article and 23.3% in the present work.

A 2014 literature review[17] compared several studies of gene expression and immunohistochemistry in women of different ages affected by breast cancer, whose results confirmed the hypothesis that young women have more aggressive molecular profiles than postmenopausal women. Other studies reached the same conclusion: young women (20–39 years) had a higher proportion of triple-negative, luminal B HER2-positive (ER+PR+HER2+, ER+PR-HER2+) and overexpressed HER2[21] tumors, while luminal A tumors predominated in those aged 40 to 98 years[21].

Analyzing the different prevalences in the two groups studied, the present study showed a higher frequency of triple-negative molecular subtypes followed by luminal B in Group I (very young women) and luminal B followed by triple-negative in Group II (young women). This result reaffirms the presence of more aggressive molecular subtypes in most young women.

Many studies show a predominance of negative hormone receptors and high rates of overexpressed HER2 tumors in young women[13,16,17,22]. In this study, we observed a slight predominance of hormone receptor-positive tumors (ER 58.89% and PR 54.44%) in the two groups analyzed and a lower percentage for overexpressed HER2 tumors (12.22%), compared to the other molecular subtypes. Perhaps the limited sample size of this study (n=90) was not enough to better assess the frequency of hormone receptors and molecular subtypes.

Regarding the CPI index, the current study demonstrated greater percentages of high CPI in triple-negative and HER2 subtype tumors in both groups, corroborating the data in the literature, which demonstrate that high Ki-67 levels are commonly associated with overexpression of HER2[23]. In addition, the literature demonstrates a correlation between hormone receptors and Ki-67, which are inversely proportional: the more positive the receptors, the lower the levels of Ki-67[23], so that the triple-negative and overexpressed HER2 subtypes, because they are hormone receptor-negative, would actually have higher levels of Ki-67.

A Norwegian study[21], published in 2019, aimed to assess the mortality rates of each molecular subtype in different age groups. The results revealed higher mortality rates in young (20–39 years) and older (70–89 years) women than in the screening-age population (50–69 years), and that triple-negative tumors were associated with higher mortality rates at all ages. The study raised the possibility that the high mortality rate in the elderly population is due to the greater number of comorbidities and less invasive treatments. On the other hand, it attributed the high death rate of young women with advanced stages of the disease at the diagnosis and high rates of more aggressive tumors[21].

Although statistical studies show that young women do not account for the highest mortality rate[3] because of the lower incidence, breast cancer in this age group is more aggressive and a reason for lower life expectancy[22].

CONCLUSIONS

The results found in this study showed a higher incidence of aggressive molecular subtypes and with a high rate of cell proliferation in young women, supporting the hypothesis that in this age group, breast cancers have a worse prognosis. Several hypotheses explain this result, such as diagnosis at an advanced stage due to lack of screening, high rates of hereditary syndromes with a high prevalence of mutations, and low clinical suspicion on the part of patients and health professionals.

The lack of individualized screening methods not only compromises early diagnosis but also prevents the adequate representation of patients with breast cancer at a young age in world surveys. Therefore, it is necessary to educate the public about the severity of the disease in young age groups, noting that even if its incidence is not high, these women have high rates of invasive tumors and metastases, and they should seek medical help through a clinical suspect condition[4].

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AUTHORS' CONTRIBUTION

RVM: Data curation, Formal analysis, Investigation, Software, Validation, Writing – original draft. YS: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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