INVASIVE TRIPLE NEGATIVE BREAST CANCER WITH BASAL AND NON-BASAL-LIKE IMMUNOPHENOTYPES: PROGNOSTIC IMPLICATIONS

Carcinoma invasivo de mama triplo negativo com imunofenótipo basal e não basal: implicações prognósticas

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ABSTRACT

Objectives: The objective of the study was to compare the postoperative staging and clinical development outcomes in a period of three years with the histopathological and immunohistochemical characteristics considered prognostic and/or predictive factors in patients being treated for triple negative type of breast cancer in the Barão Lucena Hospital, Recife, Pernambuco. Method: The study was conducted with 125 female patients suffering from triple negative breast cancer who underwent surgical treatment in the mastology service of Barão Lucena Hospital from 2009 to 2012. The clinical and pathological features of the tumors were studied and correlated with basal and non-basal subtypes. A descriptive data analysis was carried out using tables and/or graphs for qualitative variables. Association analysis was performed using χ^2 test for independence. In tables that showed expected frequency lower than 5, in more than 20% of cells, we used the Fisher's exact test. In addition, the odds ratio (OR) and the confidence interval (CI) for OR were calculated. In the entire analysis, a 5% significance level was considered. Results: Mean age was 49 years; regarding race, black was present in 83 (66.4%). The most common histological type was ductal, in 111 (88.8%). The pathological stage I/II was the most common, in 87 (69.6%) patients. A total of 71 patients (56.8%) showed no axillary metastasis. Regarding the type of surgery, the conservative one was performed in 57 (45.6%), including sectorectomy and oncoplastic surgery. The recurrence was present in 30 patients, basal in 16 (53.3%) patients and 14 (46.7%) in the non-basal, and bone metastasis was the most frequent. Conclusion: In this triple-negative tumor sample, the most important facts related to survival were: being aged less than 40 years, histological type, cytokeratin CK5/6 and higher significance level of the factors EGFR and KI-67.

KEYWORDS: Triple negative breast neoplasms; breast; breast neoplasms.

RESUMO

Objetivos: O objetivo do estudo foi correlacionar o estadiamento pós-cirúrgico e a evolução clínica em um período de três anos com as características histopatológicas e imunoistoquímicas consideradas fatores prognósticos e/ou preditivos nas pacientes em tratamento de câncer de mama do tipo triplo negativo do Hospital Barão de Lucena, Recife, Pernambuco. **Método:** O estudo foi feito com 125 pacientes do sexo feminino portadoras de câncer de mama triplo negativo e que foram submetidas a tratamento cirúrgico no serviço de mastologia do Hospital Barão de Lucena no período de 2009 a 2012. Nessas pacientes foram estudadas as características clínicas e patológicas dos tumores, as quais foram correlacionadas com os subtipos basal e não basal. A análise descritiva dos dados foi feita através de tabelas e/ou gráficos para variáveis qualitativas. Para análise de associação, foi utilizado o teste do χ^2 para independência. Nas tabelas que apresentaram frequência esperada menor que 5, em mais de 20% das caselas, foi utilizado o teste exato de Fisher. Além disso, foi calculada a razão de chance (OR) e o intervalo de confiança (IC) para OR. Em toda a análise foi considerado nível de significância de 5%. **Resultados:** A média de idade foi de 49 anos; com relação à raça, tivemos a cor negra em 83 (66,4%) delas. O tipo histológico mais comum foi o ductal, em 111 (88,8%) pacientes. O estágio patológico I/II foi o mais comum, em 87 (69,6%) delas. Um total de 71 (56,8%) pacientes não demonstrou comprometimento axilar. Com relação ao tipo de cirurgia, a conservadora foi utilizada em 57 (45,6%) pacientes, incluindo setorectomia e técnicas de oncoplastia. A recorrência esteve presente em 30 pacientes, sendo basal em 16 (53,3%) e não basal 14 (46,7%) delas, nas quais a metástase óssea foi a mais frequente. **Conclusão:** Nessa amostra de tumores triplo negativo, os fatos mais importantes associados à sobrevida foram a idade abaixo de 40 anos, o tipo histológico, a citoqueratina CK5/6 e o grau de significância maior dos fatores EGFR e KI-67.

PALAVRAS-CHAVE: Câncer de mama triplo negativo; mama; neoplasias da mama.

INTRODUCTION

Breast cancer is the global leader in terms of incidence and mortality among women. This disease has been increasing every year. In the past few years, studies have had great evolution, however, the problem is still devastating. The number of cancer cases has been growing all over the world. However, the incidence of this disease and the mortality resulting from it present relevant differences according to geographic regions[1].

The occurrence of 1 million cases of breast cancer is estimated in the next few years, and the development of the disease in more than 9% of the female population in the world. Most cases occurs in developed countries, and the Netherlands has the highest incidence (90.2/100 thousand), followed by the United States (86.9/100 thousand). About 1 out of 8 American women (about 12%) will present with invasive breast cancer throughout their lives. According to the report by the World Health Organization (WHO), global mortality in 2007 was of about 548 thousand deaths, of which 72% took place in underdeveloped or developing countries. In these countries, both the incidence and the mortality increased considerably due to population aging, changes in reproductive patterns, higher exposure to risk factors and problems related with the opportune access to early detection and diagnosis, as well as adequate treatment[2].

The early detection of breast cancer is a decisive factor in the determination of prognosis. Epidemiological studies in different Brazilian regions are important for the development of better prevention and screening programs.

They are characterized as heterogeneous neoplasms, with several histopathological subtypes, besides different clinical presentations with several variations in terms of response to treatment[3,4]. One of the major challenge for the study and treatment of the breast carcinoma is its tumoral heterogeneity[5]. The current classification of invasive breast carcinomas by the WHO is histological and presents an extensive list, of which the most common histological types are the invasive ductal carcinoma (50 to 75%) and the invasive lobular carcinoma (5 to 15%). The others, considered special types, are less frequent and include the tubular, mucinous, medullary and metaplastic carcinoma, among others[6].

The level of histological differentiation is also a measure of great utility in the clinic, and reflects the malignant potential of the tumor indicating its higher or lower capacity for metastization. The anatomopathological classification is insufficient to characterize the breast carcinomas, once tumors with the same level, stage and histological type may present with different prognosis and response to therapy[7].

There are factors involved in the prognostic evaluation of breast cancer, and it is important to consider size, type and histological grading, vascular invasion, lymphatic ganglia involvement, cell proliferation index, and expression of hormone receptors and negative epidermal growth factor (HER-2) receptors. The classification of breast carcinomas in molecular types is based on changes in some genes. With the immunohistochemical profile, we managed to obtain an indirect approximation of these subgroups, using luminal A, luminal B, HER-2 and triple negatives.

Due to the heterogeneity of the tumor types and their morphological and phenotypic traits, it is difficult to establish a relationship between the clinical findings, the prognoses and the level of recurrence that may occur with the years, after the adjuvant treatment. In our region, there are no institutional studies correlating the triple-negative tumors with the basal and non-basal like immunophenotype, with their probable prognostic implications.

METHODOLOGY

The study was carried out with 125 patients with triple-negative breast cancer of a total of 830 who were submitted to clinical and surgical treatment in the mastology service of Hospital Barão de Lucena, from July, 2009, to July, 2012. The patients were followed-up for a three-year period, when the type of treatment, recurrences and clinical and pathological characteristics of the tumors were assessed.

To interpret the negative results of HER-2, we used the 0 to 1+ score, considering as positive the tumors presenting with intense color (3+), in the entire cell membrane, in more than 30% of the assessed cells. The 0 score does not show cell membrane coloring, or is present in less than 10% of the tumor cells. Score 1+ showed slight or incomplete coloring of the membrane in more than 10% of the tumor cells[8]. The hormone receptors were considered negative when there was less than 1% and absence of expression of estrogen receptors (ER), or less than 1% of colored nuclei, according to the consensus by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP)[9].

To define the basal-like immunophenotype, we complemented the panel with the CK5/6 markers (Clone D5/16B4:DAKO) and epidermal growth factor receptor (EGFR) (Clone 31G7). According to the literature, the basal-like immunophenotype is based on the triple negativity for hormone receptors and HER-2. This hypothesis can be assessed with other markers based on clinical criterion. The core-basal designated immunophenotype (triple-negative invasive breast carcinoma positive for CK5/6 or EGFR) can be used as a predictor of the basal-like genetic molecular profile, with 76% sensitivity and 100% specificity.

The basal-like immunophenotype (CK5/6 and/or positive EGFR) was observed in 81 (64.8%) cases, and in 44 (35.2%) cases, the basal-like was not confirmed by the immunophenotype (negative CK5/6; negative EGFR). In the 81 cases with basal-like immunophenotype, 49 (60.4%) were positive for CK5/6, and 76 (93.8%) for EGFR; 44 (34.3%) presented both markers.

The descriptive data analysis was carried out using tables and/or graphs for qualitative variables. The χ^2 test of Independence was used to analyze the association. In tables presenting expected frequencies lower than 5, in more than 20% of the cells, Fisher's exact test was used. Besides, the odds ratio (OR) and the confidence interval for the OR were calculated. The 5% significance level was considered in the entire analysis. Regarding the prognostic factors determined by the immunohistochemical examination, the patients were divided in two groups: basal and non-basal. The Wald's test was used as a logistic regression model to evaluate EGFR and KI-67 according to personal and clinical factors of the patients. The tests Log Rank, Breslow and Tarone-ware were used to compare the analysis of survival, in order to obtain better statistical evaluation (Table 1).

Table 1. Distribution of frequency of the personal and clinical profiles of the patients, and contingency table with the subtype of the carcinoma.

Factor assessed	Subtype		P-value
	Basal-like (%)	Non-basal like (%)	
Age (years)			
<35	4 (100.0)	–	
35 to 45	23 (59.0)	16 (41.0)	0.175[b]
46 to 59	27 (58.7)	19 (41.3)	
60 or more	27 (75.0)	9 (25.0)	
Color			
Black	52 (62.7)	31 (37.3)	0.479[a]
White	29 (69.0)	13 (31.0)	
CK5/6			
Positive	49 (96.1)	2 (3.9)	<0.001[a]
Negative	32 (43.2)	42 (56.8)	
EGFR			
Positive	76 (97.4)	2 (2.6)	<0.001[a]
Negative	5 (10.6)	42 (89.4)	
KI-67			
Minimum	15	10	–
Maximum	90	80	–
Mean±standard-deviation	58.8±17.3	30.6±18.8	<0.001[c]
Type of surgery			
Mastectomy	45 (66.2)	23 (33.8)	
Sectorectomy	9 (64.3)	5 (35.7)	
Oncoplastic surgery	12 (57.1)	9 (42.9)	0.920[b]
Sectorectomy +AD	5 (62.5)	3 (37.5)	
Sectorectomy+SL	10 (71.4)	4 (28.6)	
Presence of metastasis			
Yes	16 (53.3)	14 (46.7)	0.131[a]
No	65 (68.4)	30 (31.6)	
Pathological staging			
I	12 (57.1)	9 (42.9)	
II	43 (65.2)	23 (34.8)	0.683[a]
III	26 (68.4)	12 (31.6)	
Histology			
Ductal	73 (65.8)	38 (34.2)	
Lobular	5 (62.5)	3 (37.5)	0,746[b]
Others	3 (50.0)	3 (50.0)	
Histological Type			
I	11 (57.9)	8 (42.1)	
II	13 (50.0)	13 (50.0)	0,002[a]
III	44 (86.3)	7 (13.7)	
Lymph nodes			
0	43 (60.6)	28 (39.4)	
1 a 3	13 (65.0)	7 (35.0)	0,615[a]
4 a 9	15 (71.4)	6 (28.6)	
10 ou mais	10 (76.9)	3 (23.1)	

EGFR: epidermal growth factor receptor; [a]p test of the χ^2 test (if p-value<0.05, the factor assessed influences the subtype of the carcinoma); [b]p-value of Fisher's Exact Test; [c]p-value of the Student's t test (if p-value <0.05, the means of both groups are significantly different); AD: axillary dissection; SL: sentinel lymph node.

RESULTS

The profiles of the triple-negative basal and non-basal like tumors were correlated with the following variables: age, skin color, histological type, pathological staging, number of compromised lymph nodes, CK5/6, EGFR, KI-67, type of surgical procedure (mastectomy, sectorectomy, with axillary or sentinel lymph node dissection, breast reconstruction) and recurrence (present or absent).

The patients were aged in average 49.77 years. The distribution of patients as to age group was: up to 35 years, 4 patients (4%); between 35 and 45 years, 39 patients (31.2%); between 46 and 59 years (36.8%); and 33 patients (28.8%) were older than 60. When these data were associated, the basal-like subtype was more prevalent in the group aged less than 35 years, and the non-basal like, in the age group of 41 to 50 years.

Regarding skin color, 52 patients (62.7%) were black, and 29 patients (69.0%) were white in the basal-like subtype. In the non-basal like subtype, the black color was found in 31 patients (37.3%), and white in 13 (31.0%).

The most common histological type was the invasive ductal carcinoma (IDC), which occurred in 111 patients (88.8%), followed by the invasive lobular carcinoma (ILC), in 8 patients (6.4%). The other types of carcinoma were medullary, tubular and mucinous, which occurred in only 6 patients (4.8%). The histological type in the basal-like subtype represented 65.8%, and in the non-basal like, 34.2%.

Regarding pathologic staging, stage I was found in 21 patients, representing 16.8%; stage II was the most present, corresponding to 66 patients (52.8%); followed by stage III< in 38 patients (30.4%). Both in basal and in non-basal like, pathologic stage II was the most prevalent one, being found in 43 (65.2%) and 23 (34.8%) patients, respectively. There was absence of axillary involvement in 71 patients (56.8%); from 1 to 3 involved lymph nodes, in 20 (16.0%); from 4 to 9 lymph nodes, in 21 (16.8%); and above 10 lymph nodes, in 13 patients (10.4%). The patients with more than ten lymph nodes compromised were associated with the basal-like subtype. In the basal and non-basal like subtypes, the absence of axillary involvement was the most present in both groups (Figure 1).

As to surgical procedure, the most frequent type of surgery was radical mastectomy (54.4%), in 68 patients. The conservative surgery with sectorectomy, using oncoplastic techniques, occurred in 57 patients (45.6%).

In the analysis of the 125 patients, we observed that in 81 (64.8%) cases, the basal-like immunophenotype was confirmed (CK5/6 and/or positive EGFR), and in 44 (35.2%), basal-like was not confirmed by the immunophenotype (negative CK5/6; negative EGFR). In the 81 cases with basal-like immunophenotype, 49 (60.4%) were positive for CK5/6, and 76 (93.8%), for EGFR (Figures 2 and 3).

The histological type, divided in three groups (I, II and III), is an important prognostic factor in triple negatives. Type I was found in 19 patients (19.8%); type II, in 26 (27.1), and type III, in 51 (53.1%). In basal-like, type III was the most frequent (86.3%), and, in non-basal like, it occurred in 13.7% (p<0.002).

We observed the adjustment of the logistic model for the subtype according to personal and clinical factors of the patients. It was observed that only EGFR and the value of KI-67 were significant together for the subtype. Also, it was observed that EGFR remains in the model as a correction factor (p=0.996), and only KI-67 was significant (p=0.022). Besides, the KI-67 growth in one unit increases the chances of the patient presenting with the basal-like subtype in 7% (Table 2).
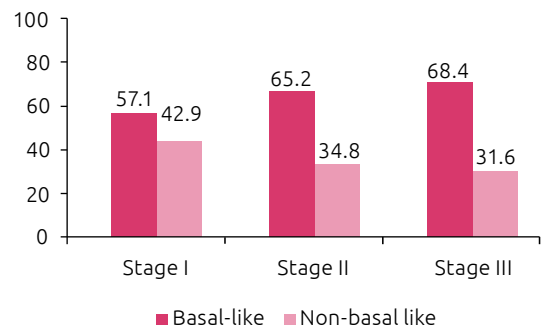

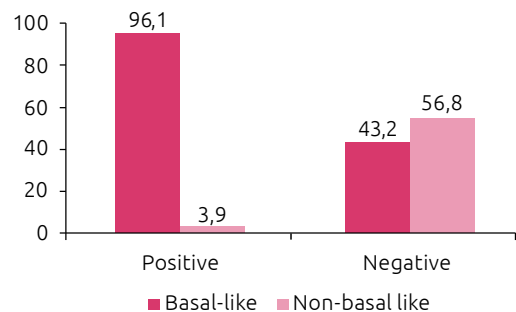**Figure 1.** Distribution of patient according to pathological staging and subtype.

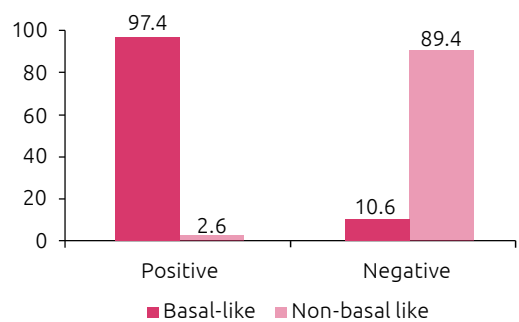**Figure 2.** Distribution of patients according to CK5/6 situation and subtype.

Figure 3. Distribution of patients according to epidermal growth factor receptor situation and subtype.

Table 3 shows the comparison tests between the personal and clinical profile of these patients and survival. It was possible to observe that color, CK5/6, type of surgery, presence of metastasis, histology and histological type are not statistically significant variables for the survival of patients in our study. The factors of age, EGFR, and pathologic staging were significant to determine the survival of the patient, who were assessed for 36 months.

The survival curve shows these data. It is important to mention that the presence of metastasis and the histological type were very close to significance in the three tests applied, indicating there is a tendency of these variables to influence on the survival of the patient (Figures 4, 5 and 6).

Regarding recurrence, 30 (24.0%) patients had metastasis, of whom 16 belonged to the basal-like group (53.3%), and 15 (46.7%), to the non-basal like group. Thirty patients presented with metastasis (16.26%), and 95 patients did not (83.74%). In patients with metastasis, the bone form was the most common, in 11 patients

Table 2. Logistic model for the subtype according to personal and clinical factors of the patients.

Factor assessed	OR	CI	P-value[a]
EGFR			
Positive	5.58×10^9	*	0.996
Negative	1.00	−	
KI-67	1.07	1.01–1.13	0.022

OR: *odds ratio*; CI: confidence interval; EGFR: epidermal growth factor receptor; [a]p-value of the Wald's test (if p<0.05, the assessed factor is determinant for the basal-like subtype); *it was not possible to calculate the confidence interval.

Table 3. P value of the survival comparison tests between the personal and clinical profile of the assessed patients.

Factor assessed	P value of the test			
	Log rank	Breslow	Tarone-ware	Wald
Age	0.010	0.003	0.005	−
Color	0.211	0.125	0.160	−
CK5/6	0.137	0.146	0.140	−
EGFR	0.024	0.027	0.025	−
KI-67	−	−	−	0.405
Type of surgery	0.657	0.667	0.659	−
Presence of metastasis	0.083	0.040	0.056	−
Pathological staging	0.036	0.038	0.036	−
Lymph node involvement	0.632	0.690	0.670	−
Histology	0.134	0.073	0.097	−
Histological type	0.410	0.410	0.410	−

EGFR: epidermal growth factor receptor.

Age

- <35 years
- 35 to 45 year
- 46 to 59 years
- 60 years or more
- <35 years-censored
- 35 to 45 years-censored
- 46 to 59 years-censored
- 60 years or more-censored
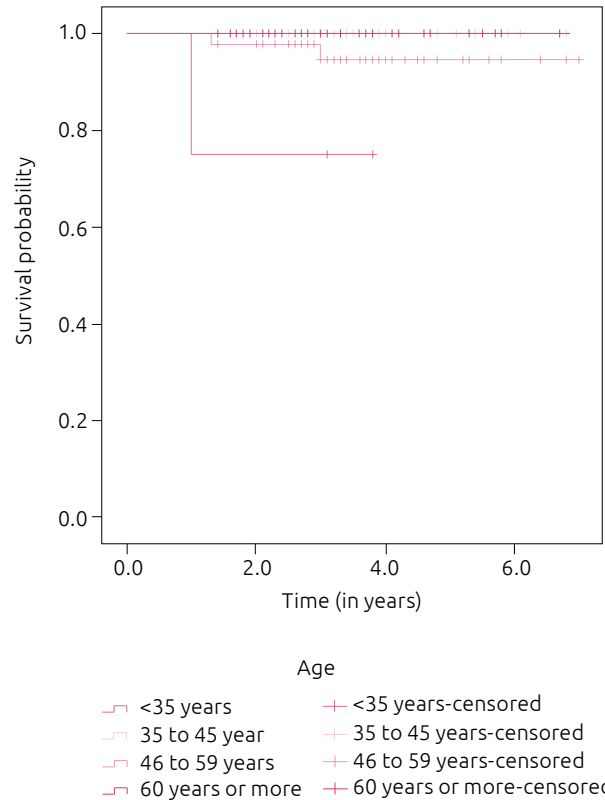
Figure 4. Analysis of survival of the patients according to age group.

Epidermal growth factor receptor

- Positive
- Negative
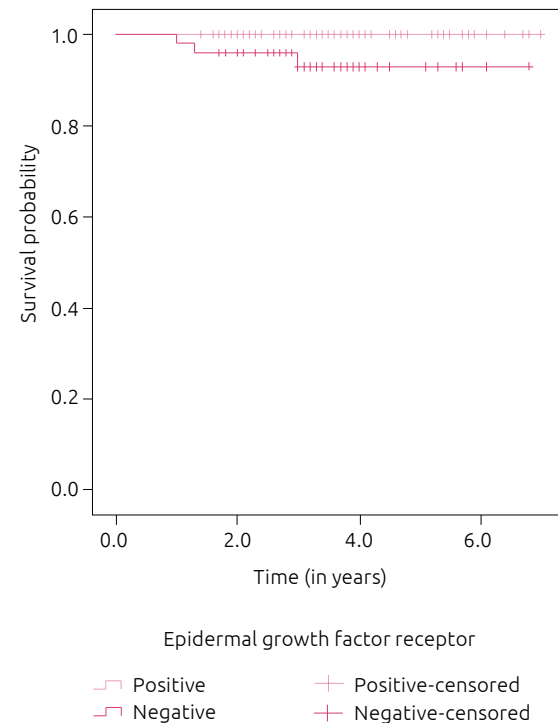- Positive-censored
- Negative-censored

Figure 5. Anaysis of survival of patients according to epidermal growth factor receptor.

(5.2%); followed by pleural-lung metastasis, in 6 patients (2.84%). There were two deaths in the three-year follow-up period, one caused by meningeal and brain metastasis.

The figures show the graphic representation of the factors that had the most statistical significance for the evaluated patients.

DISCUSSION

The natural history of triple-negative breast is not clear, since its evolution does not take place in a uniform manner in all women, being very heterogeneous. Because of this behavioral divergence in relation to some tumors that present the same clinical characteristics, it is important to analyze more prognostic factors that involve its general context. Triple-negative tumors have different epidemiological characteristics, as well as the behavior and evolution of the basal-like and non-basal like subtype. Therefore, besides the diagnosis of the disease itself, there are clinical and biological aspects associated with the differences of disease-free and global survival time[10,11].

The appropriate identification of prognostic factors and the predictive responsive parameters to a specific treatment are still challenges in the individualization of the best therapy for each patient[12]. A series of recommendations for the selection of adjuvant

Figure 6. Analysis of survival of patients according to pathological staging.

systemic therapies for triple-negative basal and non-basal like tumors has been recently proposed in the 14th International Conference on Adjuvant Therapy of Primary Breast Cancer, in Saint Gallen, Switzerland[13].

The increased expression of some of these biological substances can be present in malignant breast neoplasms, presenting relevance from the prognostic point of view. The most important biological factors are the estrogen and progesterone hormone receptors, the expression of the anomalous protein p53 and the amplification of the oncogene HER02, as is the absence of hormone and HER-2/neu protein receptors[14].

The patient's age at the time of diagnosis is important, since it is directly related with the menopausal status, and, consequently, with the hormone action in tumor growth. Older women, in post-menopause, with breast cancer present increasing concentration of ER in the tumor, and their cancers are usually well differentiated, with low rates of proliferation[15]. However, in younger women, it is common to find triple-negative and poorly differentiated tumors. In our study, we observed that mean age was 45 years, in a pre-menopausal stage, in which 46 patients were aged between 46 and 59 years, above the average found in the literature, which presents higher frequency below the age of 40. Van Belle et al.[16], in a survey, showed mean age of 40 years. Studies demonstrated that younger women have worse prognosis in relation to older women, in post-menopause[17]. Large tumors, ganglion involvement, negative for ER, increased phase S and p53 abnormality are very common at the age of 30 to 35 years[15]. In our sample, patients aged less than 40 years, in 27 cases, represented 33.7% of the sample, showing increasing presence of breast cancer in younger patients, possibly due to changes in the lifestyle of the modern woman[18]. Studies with multivariate analysis concluded that younger women have worse prognosis, with increasing risk of recurrence and death[19]. The correlation with the basal and non-basal like subtype presented non-significant p in the examined patients. In our sample, there was no change in prognosis in the two triple-negative subtypes (p=0.175); however, in the studies by Slamon et al.[20], Dati et al.[21], Clark[22] and in the analysis by Farzadnia et al.[23], there was no correlation between age and hormone receptor factors and the HER-2 protein.

Regarding race, the literature shows that women with African American ancestors are in a more advanced stage, with higher mortality index in comparison to white women[24]. Other authors have shown that black or Hispanic patients present with advanced tumors, positive lymph nodes at diagnosis and factors indicating more biological aggressiveness, with negative receptors and high level of proliferation[25]. A higher number of cancer cases is observed in black women, in comparison to white women, in patients aged less than 40 years, and normally present with higher nuclear grade, do not usually have hormone receptors, are
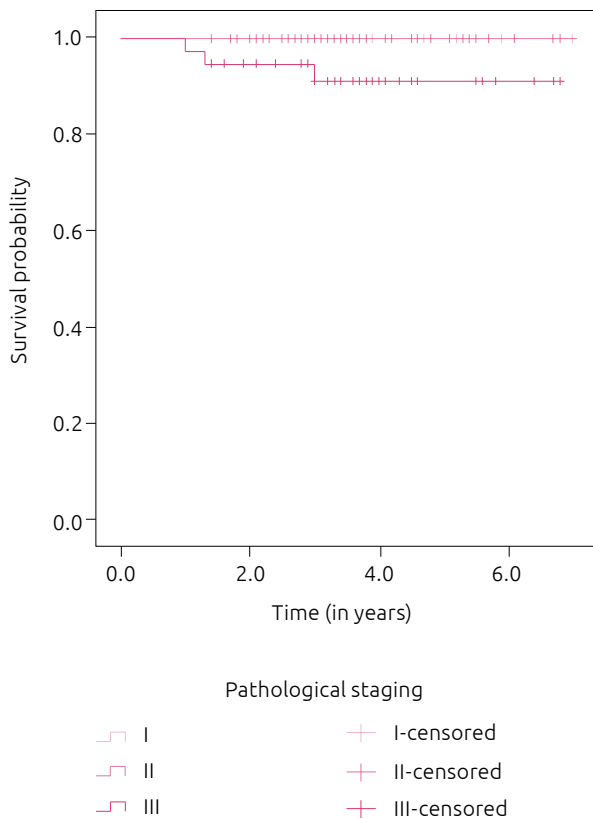
negative for the HER-2 protein and suffer with different types of sporadic p53 mutations[26]. In our study, the black race was mostly affected, constituting 83 cases (66.4%). There is high prevalence of triple-negative tumors in young, black patients[27]. Our sample showed that triple-negative tumors in black patients were found, as the basal-like subtype, in 52 of them (62.7%), and non-basal like, in 31 patients (37.3%), presenting higher prevalence in the white patients. A study conducted in our country by Carvalho et al. in 2014[28] found differences in the molecular subtypes in the several regions of Brazil. The North region had higher incidence of triple-negative (20.3%), with higher African influence (77.8%), when compared to the Northeast (65.5%), and the Center-West (65.9%). In the South and Southeast regions, this incidence is lower.

The invasive ductal carcinomas have worse prognosis, higher incidence of axillary involvement, and constitute most breast invasive carcinomas, corresponding to about 80% of the cases[29]. In our sample, the ductal histological type was found in 111 patients (88.8%). The 30-year survival rate for women with special types of invasive carcinomas (tubular, mucinous, medullary, lobular and papillary) is higher than 60% in comparison to less than 20% in women with the common invasive ductal carcinoma[29]. Among the special histological types, the pure papillary, tubular and mucinous carcinomas, which appear in approximately 2% of the cases, present with the best prognosis[30]. In the sample, the special types were found in only 6 patients (4.8%). The invasive lobular carcinoma, which appears in approximately 10% of the cases, presents different biological and clinical characteristics, since it affects older patients; it is a larger tumor, with higher immunoexpression for ER or progesterone receptor (PR), associated with fraction of the low S phase, with negative HER-2, p53 and EGFR[31,32]. The lobular carcinoma was found in 8 patients (6.4%), but, due to the small number of cases, it was not possible to verify an association with the basal and non-basal like subtypes. There is a direct correlation between tumor size, axillary involvement and level of recurrence of the disease. The tumor diameter is the second prognostic factor in level of importance, and is independent of the condition of the lymph node[28]. Many studies have shown a relationship between tumor size and survival[33]. The survival rate of women with tumors smaller than 1 cm in 10 years is approximately 90%. On the other hand, more than half of the women with tumors larger than 2 cm will have higher chances of lymph node involvement, and many will die[28]. The mean size found in our sample was 3.79 cm, similarly to what was found by Uemura et al[35]. The stage II tumors were found in 96 patients (46.19%) and those considered as stage I, in 62 patients (29.04%). It is possible that tumors that grow too fast in size, without lymph node metastasis, have low chances of distant metastasis[36,37]. We can observe that more than 70% of the tumors were found at initial stages, which enabled conservative, less mutilating surgeries. In a different direction, Bacha et al.[38] found a relationship of the different immunophenotypes (Re, Rp and HER-2) with sizes of tumors III and IV.

Regarding pathological staging, stage I was found in 21 patients (16.8%), stage II corresponded to 66 patients (52.8%), and stage III, in 38 (30.4%). By correlating the basal and non-basal like subtypes, no relationship was found. On the other hand, Mattes et al. in 2014[37] found stage I in 67.2%, followed by stage II, with 286%, in different molecular subtypes. The observation was that triple-negative tumors have low risk of axillary involvement, unlike positive hormone receptors and HER-2, which have high rates of lymph node involvement. Farzadnia et al.[23] did not find correlation between the worst tumor stage and the immunophenotype of HER-2. In the studies by Yuan[39] and Imoto[40], there was correlation between tumor size and proto-oncogene HER-2. In another survey, carried out by Richi and Junqueira[41], there was no statistically significant relation between morphological (tumor size, type and histological grading) or molecular factors (ER/PR/HER-2/KI-67/p53) and the occurrence of metastasis. They established there is a continuous relationship between slow-growing tumors, late axillary lymph node involvement, and more aggressive tumors, with early metastasis to the axilla[42].

The occurrence of metastasis in axillary lymph nodes is considered the most powerful and independent parameter of breast cancer prognosis, in the absence of distant metastasis[43,44]. Axillary involvement is indication for adjuvant systemic therapy. In patient with negative axillary lymph nodes, it is important to assess other prognostic factors, such as tumor size, histological grading, hormone receptors, patient's age and some cell proliferation markers to determine therapy[45]. When the lymph nodes are free of neoplasm, the disease-free survival rate in 10 years is of approximately 70 to 80%; in patients with 1 to 3 involved lymph nodes, it is 35 to 40%; and those with more than 10 lymph nodes involved, it is 10 to 15%. The 5-year survival for patients with negative axilla is 82%, in comparison to 45% for patient with 4 to 12 involved lymph nodes, according to the National Surgical Adjuvant Breast and Bowel Project (NSABP). The use of a sentinel lymph node biopsy is an alternative for the axilla approach, without increasing the costs and preventing the complications of axillary dissection[43]. Regarding the nummber of lymph nodes involved in our survey, most of our patients, 71 (56.8%) did not have axillary involvement; followed by 1 to 3 lymph nodes involved, in 20 patients (16.0%). In those patients who had between 4 and 9 lymph nodes involved, 21 (16.8%), and in patients with more than 10 lymph nodes, 13 (10.4%); there was no association with the basal and non-basal like subtypes. Bacha et al.[38] verified, in patients who had more than 10 lymph nodes involved, a correlation with positive HER-2, with statistically significant finding (p=0.044). In the studies by Wang et al.[46] and Curiliano et al.[47], there was significant relation between the cases with negative receptors and positive HER-2 and positive axilla (p=0.039).

We observed that the mastectomy surgery was used in 68 patients (54.4%), conservative surgery with sentinel lymph node or axillary dissection, in 36 patients (28.8%), and oncoplastic techniques, in 21 (16.8%). These data demonstrate the occurrence of more mutilating surgeries, however, with very interesting balance between mastectomy and conservative surgery in our area, especially due to the increasing rates of early diagnosis of breast cancer. The use of oncoplastic techniques, in many cases, aims at the minimum treatment with maximum effect, always respecting the principles of the classic oncological surgery. With these results, we believe that, in the future, conservative surgeries will overcome radical ones, with the use of oncoplastic techniques. Differently, in the study by Vallejos et al.,[48] with a total of 1,198 patients with breast cancer, about 70 to 75% of the them had radical mastectomy, and the rest underwent conservative surgery. In the correlation between the basal and non-basal like subgroup with the type of surgery, there was no statistical significance.

The dissemination of breast cancer is a common situation, because, from the time of diagnosis, about 5% of the patients present with distant conditions, according to the National Cancer Statistic Review[49]. The most common places of metastization are soft parts, bones, liver, and lungs[50]. In the metastatic phase, the disease always presents an unfavorable prognosis, with mean survival ranging from 24 and 42 months, and mean survival rate in 5 to 10 years. During the period of evaluation of our sample, all patients were followed-up and assessed in the follow-up of the presence or absence of distant metastasis. There was metastasis in 30 patients, which represented 24%, and absence in 95 patients (76%). In patients with metastasis, the bone type was more common, in 14 patients (46.6%), followed by lung and liver, with 5 patients for each, in a total of 10 patients (33.3%). In an interesting survey by Koo et al.[51], in 34 cases of metastatic cancer, the liver lesions, ER/PR+ and HER-2 were more prevalent; in brain metastasis, the overexpression of HER-2 was the most common one. In bone lesions, triple-negative tumors were prevalent. Bollen et al.[52] observed that patients with bone metastasis in the triple negative had mean survival of 6.7 months, unlike patients with positive receptors, whose survival was 22.5 months. In our survey, there was no correlation between the presence or absence of metastasis and the basal and non-basal like group.

Together with the classic parameters, like tumor size staging, axillary nodule and metastasis (TNM) and cell proliferation markers, other tumor markers have been used to predict tumor behavior, and the response to therapy in breast cancer. Among the markers verified by the immunohistochemical test, the most used in clinical practice are hormone receptors and HER-2[52]. The normal breast cell presents estrogen and progesterone receptors in their nuclei, detected through the immunohistochemical method. The level of receptors for steroid, estradiol (RE) and/or progesterone (PR) hormones in the tumor tissue constitutes strong indication of the level of hormone dependence in the mammary neoplasm[7]. Women with positive cancers for hormone receptors have prognosis slightly better than women with carcinomas that are negative to hormone receptors[53]. The evaluation of hormone receptors is more valuable to predict response to therapy, and hormone therapy usually has an excellent response in positive cases, with little or no response to this type of treatment in negative cases[54]. The overexpression of HER-2 is associated with poor prognosis, however, its evaluation has been mostly used to determine response to specific therapy. The triple-negative tumors represent about 15% of the total breast tumors, and affect younger patients more often, usually before menopause[55]. Vallegos et al.[48], in a survey including 1,524 patients, verified that approximately half of all cases were classified as positive ER/PR, and negative HER-2 (Luminal A subtype), corresponding to 49.3%; about 13.2% were identified as positive ER/PR/HER-2, and tumors in the triple-negative subtype (negative ER/PR/HER-2) were observed in 21.4% of the patients, demonstrating a very similar result to that found in our study. Wang et al.[46] found the triple-negative in 16.9% of the 835 patients with breast cancer, and these patients are more prone to developing distant metastasis.

In a recent review, Carter et al.[34] found prevalence of the high level of HER-2, between 0 and 38%, with mean of 18%, in women with breast cancer. In this study, we found 32% of positivity in the patients.

Our molecular biology techniques allow determining the true genetic signatures of the tumor by analyzing thousands of genes simultaneously. There is a new era of prognostic and predictive factors in the response to breast cancer treatment[54]. These tumors are classified in five subtypes: luminal A, luminal b, normal epithelium, basal-like and overexpression of HER-2[55,56]. We observed that tumors with good prognosis are luminal A and B and normal epithelium, whereas the evolution is worse in the basal-like and in the overexpression of HER-2 types. Even though there has not been a study about genetic signature in our analysis, we tried to conduct a classification of the groups based on the immunohistochemical criteria. There are studies that use immunohistochemistry in the subdivision of these genetic groups; however, further prospective, large studies are necessary to verify the correlation between these findings and genetic classification[57]. The genetic signature method has brought more independent prognostic information in relation to clinical-pathological prognostic factors[58,59].

After the immunohistochemical analysis of EGFR, cytokeratin CK5/6 and KI-67, we observed that these markers are associated with triple-negative breast carcinomas. This type of basal-like carcinoma is distinguished by the expression of keratins, which are more typical of myoepithelial cells. EGFR was overexpressed in 76 patients (97.4%), which is very characteristic in the basal-like subtype in triple-negatives, which was significant. Our results

also showed that triple-negative tumors have higher expression of the proliferation of KI-67 in the basal-like subtype, with 58.8%, than in the non-basal like subtype, with 30/6%, which is associated with worse prognosis in the evolution of the disease, normally presenting score higher than 15. In the statistical test, the KI-67 factor was significant, indicating that, in average, the value of this marker is different between the group of basal and non-basal like patients. Even with this prevalence in these groups, the independence test was significant only in factors CK5/6, EGFR and histological type, indicating that these factors are determinant for this subtype of the carcinoma. Kanapathy et al.[60] observed, in 340 patients diagnosed with breast cancer, that 12.4% (42) were triple-negative and were strongly associated with EGFR, CK5/6, and high level of KI-67 proliferation.

The patients who underwent neoadjuvant and adjuvant chemotherapy in triple-negative cancer, as well as radiotherapy, which was carried out at the location and on the supraclavicular fossa, from 25 to 30 sessions. Neoadjuvant chemotherapy was indicated in cases of locally advanced tumors. The anthracycline-taxane combination was chosen for 4 to 6 months, and, in this group, showed the best pathological complete response (PCR) in the breast and axilla, of 43.2%, for patients with negative hormone receptors. It has been consistently demonstrated that PCR is a very good prognostic factor for long-term benefits, especially for triple-negative patients[61,62], who, in general, reach 28 to 32%

rates of PCR[61]. The anthracycline-taxane combination should be, whenever possible, chosen due to the best rates of PCR[63,64]. There is experimental evidence that the addition of salts of platinum can be beneficial for triple-negatives, especially for patients with BRCA1 mutation[65,66].

Nowadays, the study of molecular biology, including the genetic signature, has been important to define the therapeutic sensitivity and to study small tumors better, free axilla, and considered, mostly, of good prognosis, may present with early recurrence; others, sometimes considered to be complicated, have favorable evolution. The evaluation of the triple-negative basal and non-basal like molecular subtype is very important to identify patients with worse prognosis. It is important not to base a diagnosis exclusively on the results of the immunohistochemical reactions. It is necessary to compare them with the histopathological findings and with the clinical data and imaging of the case.

CONCLUSIONS

The triple-negative basal and non-basal like tumors are different entities. In the three-year follow-up, the prognostic factors that mostly influenced the basal and non-basal like subtype were age, in young patients, histological type, and cytokeratin CK5/6, with higher significance level in factors EGFR and KI-67.

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