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Clinical, histomorphological, and therapeutic prognostic factors in patients with triple-negative invasive breast cancer

Fatores prognósticos clínicos, histomorfológicos e terapêuticos em pacientes com câncer de mama invasivo triplo negativo

Camila M. Lopes; Mário R. Montemor Netto; Fábio P. Mansani; Rebecca S. M. Stival; Maíra R. Cassapula; Tainá Fernanda B. Oliveira

Universidade Estadual de Ponta Grossa (UEPG), Paraná, Brasil.

ABSTRACT

Introduction: Breast cancer is the most common visceral malignancy in women, the leading cause of cancer death among women worldwide. The triple negative subgroup has poor prognosis and aggressive biological behavior. **Objectives:** To outline the clinical and histopathological aspects, the treatment profile, and to suggest which factors may predict poor prognosis in patients with triple-negative invasive breast cancer in the Campos Gerais region of Paraná. **Methods:** A retrospective observational cohort study, longitudinal, comparative, performed in a clinic of anatomic pathology in the Instituto Sul Paranaense de Oncologia, in Ponta Grossa, Paraná. The inclusion criteria were female patients with pathology report of invasive breast carcinoma, whose immunohistochemistry showed negative for hormone receptors and human epidermal growth factor receptor (HER2), diagnosed in the period between January 1, 2002 and December 31, 2012. The patients were divided into two groups, living women and patients who have died. **Results:** The recurrence rate, chemotherapy type, angiolymphatic invasion, tumor size, lymph node invasion, and type of surgery performed were significant variables in the univariate analysis between the groups. After Cox regression for multivariate analysis, only the angiolymphatic invasion ($p = 0.012$, relative risk [RR] 5.0518, confidence interval [CI] 95% 1.4261-17.8952), and tumor size ($p = 0.0385$, RR 1.2605, CI 95% 1.0123-1.5695) remained significant. **Conclusion:** The angiolymphatic invasion and tumor size proved to be risk factors for death, from all causes, in patients with triple-negative breast cancer. Differences between groups can indicate different molecular subtypes within the triple-negative phenotype.

Key words: breast cancer; immunohistochemistry; prognosis; death.

INTRODUCTION

Breast cancer (BC) is the type of visceral malignancy that more affects women, representing 25% of all cancers diagnosed in this gender; it is the leading cause of cancer death among women worldwide. For Brazil, in 2014, it was expected 57.120 new cases of BC, with an estimated risk of 56.09 cases per 100,000 women[1].

BC is a heterogeneous disease, involving several molecular subtypes associated with different morphological characteristics and clinical behavior. BC may be differed according the following profiles, through genetic microarray analysis: luminal A, luminal B, normal breast-like, with human epidermal growth factor receptor 2-overexpressing (HER2), and triple negative (TN)[2], each with a different prognosis.

The TN subgroup has poor prognosis and aggressive biological behavior. This corresponds to 10%-20% from all cases of primary BC and is defined, by immunohistochemistry, due to the absence of expression of estrogen receptor (ER), progesterone receptor (PR) and HER2[3]. It is characterized by affecting younger women and have high risk of recurrence, lower disease-free survival and lower overall survival[4]. Histopathological aspects are presented unfavorable with high histological grade, high mitotic count and marked nuclear polymorphism[5]. This type of cancer do not have targeted therapy and is insensitive to hormone therapy, but shows good response to chemotherapy[2].

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Therefore, in order to contribute with more knowledge on this malignancy to literature, this study sought to identify clinical and histomorphological features and the treatment performed, as well as to indicate the risk factors for mortality, from all causes, in patients affected by triple negative breast cancer (TNBC).

METHODS

Retrospective observational cohort study, longitudinal, comparative, performed by pathological analysis of anatomic-pathology and immunohistochemical reports of a private clinic of anatomic-pathology and the medical records from the Instituto Sul Paranaense de Oncologia, in Ponta Grossa, Paraná, in patients diagnosed with invasive TNBC during the period of 2002-2012.

The study was approved by the Human Research Ethics Committee (HREC) of the Universidade Estadual de Ponta Grossa (UEPG).

Patients

Inclusion criteria for the studied sample were female patients with anatomic-pathology report of invasive breast carcinoma, whose immunohistochemistry (IMH) was negative for ER, PR and HER2, diagnosed in the period from 1 January 2002 to 31 December 2012. We excluded those who had insufficient data in anatomic-pathology or immunohistochemical reports.

The tumors were evaluated for the presence of hormone receptors (estrogen and progesterone) and HER2 overexpression. These markers were tested by IMH and interpreted according to the College of American Pathologists recommendations[6]. The evaluation of ER and PR was considered positive when ≥ 1% of neoplastic cells exhibited immunoreactivity, and negative when in < 1% of these. For assessment of HER2 the expression of the protein in the membrane of tumor cells was considered. The zero value (negative) corresponds to the cases where no staining is observed or membrane staining is present in less than 10% of tumor cells; 1+ (negative), weak staining is detected in more than 10% of tumor cells, with only part of the membrane of tumor cells stained; 2+ (equivocal) weak to moderate staining is observed along the full-length of the membrane in more than 10% of tumor cells or intense complete staining, all along the membrane, but ≤10% of invasive tumor cells, and 3+ (strongly positive) moderate to strong staining is observed along the length of the membrane in more than 10% of tumor cells. In HER2 equivocal cases, the FISH (fluorescence *in situ* hybridization) test was performed to determine the presence or absence of gene amplification. Positive FISH results were considered positive for HER2. In this study, the indeterminate cases without FISH analysis were excluded.

Included and analyzed variables

The clinical characteristics studied were: age at diagnosis, time of follow-up, reason for appointment, time from the first symptom/signal until appointment with the doctor, age at menarche and menopause, age at first pregnancy, parity, breastfeeding, use of hormone replacement therapy (HRT) and hormonal birth control (HBC), family history of breast and/or ovarian cancer, alcoholism, smoking, comorbidities, body mass index (BMI), clinical stage at diagnosis and relapse. The time of follow-up, calculated in months, corresponds to the time elapsed from diagnosis to outcome, determined by the latest appointment or the date of death. The time of the first symptom/sign until appointment to the doctor in months corresponds to the time period since the onset of symptoms to the health care demand. The age of first pregnancy corresponds to the patient's age at her first child birth. Breastfeeding was calculated in cumulative months throughout that period the patient reported having breastfed. Both use of oral HRT and HBC were positive when the patient reported having used at certain moment in their life. Family history was positive when referred in first, second or third -degree relatives. Both alcoholism and smoking were positive when the patient mentioned such addiction at some moment in their life. Comorbidities were defined as diseases present at the diagnosis of BC. The initial clinical stage at diagnosis followed the T: tumor, N: lymph node, M: metastasis (TNM) classification of the Union for International Cancer Control (UICC)[7]. Relapse was defined at the time of neoplasia recurrence regardless of location.

The anatomic-pathological variables included were: histological type and tumor grade, pathological staging (pTNM), Nottingham histological score, angiolymphatic invasion, perineural invasion, skin and nipple invasion. All these criteria were established based on the protocol for examination of samples from patients with invasive breast carcinoma, by the College of American Pathologists[8].

The types of treatment observed were surgery, radiotherapy and chemotherapy.

Stratified sample

The patients were divided into two groups, women alive (cases, $n = 64$) and patients who died (control, $n = 33$), according to the latest information in the medical record. In death, we considered death from all causes.

Clinical and morphometric characteristics of BC and treatment of patients studied are shown in **Tables 1**, **2** and **3**.

TABLE 1 – Clinical characteristics of the triple-negative breast cancer

Variables	*n* (%)
Age at diagnose	
Mean ± SD (years)	54.80 ± 14.10
Total	97 (100)
Patients' status	
Living	64 (65.98)
Deaths	33 (34.02)
Total	97 (100)
Age at death	
Mean ± SD (years)	57.33 ± 13.37
Total	33 (100)
Time of follow-up	
Median (IQR) (months)	29 (15-72)
Total	97 (100)
Reason for appointment*	
Palpable breast mass	48 (64)
Mastalgia	22 (29.33)
Breast swelling	17 (22.66)
Abnormal mammogram result	12 (16)
Axilla/upper limb complaints	7 (9.33)
Breast pain	5 (7.81)
Hardening of breast tissue	4 (5.33)
Nipple retraction	3 (4)
Wound in breast	2 (2.67)
Others	3 (4)
Total	75 (100)
Time from 1st symptom until appointment with the doctor	
Median (IQR) (months)	6 (3-12)
Total	60 (100)
Menarche	
Median (IQR) (years)	13 (12-14)
Total	57 (100)
Menopause	
Median (IQR) (years)	48 (44-51.5)
Postmenopausal women	61 (87.14)
Premenopausal women	9 (12.86)
Total	70 (100)
Age at 1st child birth	
Mean ± SD (years)	21.87 ± 3.44
Total	49 (100)
Giving birth	
Median (IQR) (pregnancies)	3 (2-5)
No pregnancy	11 (15.71)
One or more pregnancies	59 (84.29)
Total	70 (100)
Breastfeeding	
Median (IQR) (months)	9 (6-40)
Yes	11 (64.71)
No	6 (35.29)
Total	17 (100)
Use of HRT	
No	14 (70)
Yes	6 (30)
Total	20 (100)
HBC	
Never used	24 (64.86)
Had already used	13 (35.14)
Total	37 (100)
Family history of breast and ovarian cancer	
No	54 (72)
Yes	21 (28)
Total	75 (100)
Alcoholism	
No	56 (94.92)
Yes	3 (5.08)
Total	59 (100)
Smoking	
Never smoked	62 (74.70)
Had already smoked	21 (25.30)
Total	83 (100)
Comorbidities	
Without comorbidities	24 (28.92)
HBP	26 (31.33)
DM	11 (13.25)
Dyslipidemia	9 (10.84)
Hypothyroidism	7 (8.43)
Osteoporosis	6 (7.23)
Total	83 (100)
BMI	
Median (IQR) (kg/m^2)	25 (22-29)
Total	79 (100)
Clinical staging	
0	0 (0)
I	7 (7.37)
IIA	24 (25.26)
IIB	21 (22.11)
IIIA	14 (14.74)
IIIB	25 (26.32)
IIIC	1 (1.05)
IV	3 (3.16)
Total	95 (100)
Recurrence	
Yes	35 (53.03)
No	31 (46.97)
Total	66 (100)

**The percentages may be greater than 100%, since patients may have more than one complaining for appointment.*
SD: standard deviation; IQR: interquartile range; HRT: hormone replacement therapy; HBC: hormonal birth control; HBP: high blood pressure; DM: diabetes mellitus; BMI: body mass index.

TABLE 2 – Histomorphological characteristics of triple-negative breast cancer

Variables	*n* (%)
Histological type	
Invasive ductal, NOS	91 (93.81)
With apocrine differentiation	3 (3.09)
Metaplastic of no special type	2 (2.06)
Medullary	1 (1.03)
Total	97 (100)
Angiolymphatic invasion	
Present	60 (61.86)
Absent	37 (38.14)
Total	97 (100)
Perineural invasion	
Present	16 (16.49)
Absent	81 (83.51)
Total	97 (100)
Skin invasion	
Present	14 (16.28)
Absent	72 (83.72)
Total	86 (100)
Nipple invasion	
Present	6 (8.22)
Absent	67 (91.78)
Total	73 (100)
Nottingham histological score	
Nuclear pleomorphism	
Low grade	0 (0)
Intermediate	5 (5.15)
High grade	92 (94.85)
Total	97 (100)
Tubular differentiation	
> 75%	0 (0)
Between 10% and 75%	39 (40.21)
< 10%	58 (59.79)
Total	97 (100)
Mitotic index	
≤ 3 mitosis/mm²	42 (43.30)
4-7 mitosis/mm²	37 (38.14)
≥ 8 mitosis/mm²	18 (18.56)
Total	97 (100)
Histological grade	
I	2 (2.06)
II	51 (52.58)
III	44 (45.36)
Total	97 (100)
Pathological staging	
Primary tumor (size)	
pTX	1 (1.09)
pT0	0 (0)
pTis	0 (0)
pT1mi	0 (0)
pT1a	1 (1.09)
pT1b	3 (3.26)
pT1c	17 (18.48)
pT2	48 (52.17)
pT3	10 (10.87)
pT4a	0 (0)
pT4b	7 (7.61)
pT4c	0 (0)
pT4d	5 (5.43)
Total	92 (100)
Lymph node invasion	
pNX	1 (1.09)
pN0	47 (51.09)
pN1mi	0 (0)
pN1a	25 (27.17)
pN2a	13 (14.13)
pN3a	6 (6.52)
Total	92 (100)

NOS: not otherwise specified.

TABLE 3 – Treatment of triple-negative breast cancer

Variables	*n* (%)
Surgery	
Modified radical mastectomy	62 (67.39)
Breast-conserving surgery	23 (25)
Halsted radical mastectomy	5 (5.43)
Total or simple mastectomy	2 (2.17)
Total	92 (100)
Radiotherapy	
Only adjuvant radiotherapy	61 (64.89)
Only palliative radiotherapy	9 (9.58)
Adjuvant and palliative radiotherapy	1 (1.06)
Did not receive radiotherapy	23 (24.47)
Total	94 (100)
Chemotherapy	
Neoadjuvant chemotherapy	7 (7.45)
Adjuvant chemotherapy	49 (52.13)
Palliative chemotherapy	5 (5.32)
Neoadjuvant and adjuvant chemotherapy	10 (10.64)
Neoadjuvant, adjuvant and palliative chemotherapy	5 (5.32)
Neoadjuvant and palliative chemotherapy	5 (5.32)
Adjuvant and palliative chemotherapy	9 (9.57)
Did not receive chemotherapy	4 (4.25)
Total	94 (100)

Statistical analysis

Statistical analysis was performed using the MedCalc software, version 13.1.2. Samples of quantitative variables were tested for normality with the Shapiro-wilk test (cut line $p > 0.05$) and its comparison, performed by Student's *t*-test and Mann-Whitney test. The comparison of categorical variables was performed using Fisher's exact test and the chi-square test. Cox regression used four significant variables, time of follow-up and final outcome. Statistical significance was considered at $p < 0.05$.

All information was collected after the HREC of UEPG approval.

RESULTS

From the analysis of anatomic-pathological reports, 97 cases met the criteria for inclusion in the study. According to the records, living female patients totaled 64 (65.98%), and 33 (34.02%) the patients who died.

Clinical characteristics

The data are presented in **Table 4**.

TABLE 4 – Clinical characteristics of the triple-negative invasive breast cancer distributed by outcome

Variables	Living *n* (%)	Deaths *n* (%)	*p* value
Age at diagnose			
Mean ± SD (years)	54.92 ± 14.63	54.58 ± 13.24	
Total	64 (100)	33 (100)	0.9095
Reason for appointment*			
Palpable breast mass/lump	33 (66)	15 (60)	
Mastalgia	16 (32)	6 (24)	
Breast swelling	10 (20)	7 (28)	
Abnormal mammogram result	6 (12)	6 (24)	
Axilla/upper limb complaints	5 (10)	2 (8)	
Breast pain	3 (6)	2 (8)	
Hardening of breast tissue	4 (8)	0 (0)	
Nipple retraction	2 (4)	1 (4)	
Wound in breast	1 (2)	1 (4)	
Others	1 (2)	2 (8)	
Total	50 (100)	25 (100)	0.7158
Menarche			
Median (IQR) (years)	13 (12-14)	13 (12-14)	
Total	38 (100)	19 (100)	0.8625
Menopause			
Median (IQR) (years)	48 (45-51)	50 (42-52)	
Postmenopausal women	44 (88)	17 (85)	
Premenopausal women	6 (12)	3 (15)	
Total	50 (100)	20 (100)	0.9614
Age at 1st child birth			
Mean ± SD (years)	21.96 ± 3.30	21.67 ± 3.87	
Total	34 (100)	15 (100)	0.8099
Giving birth			
Median (IQR) (pregnancies)	3 (1-4)	3 (1-5)	
No pregnancy	8 (16.67)	3 (13.64)	
One or more pregnancies	40 (83.33)	19 (86.36)	
Total	48 (100)	22 (100)	0.7352
Breastfeeding			
Median (IQR) (months)	6 (0.5-19.5)	0 (0-19)	
Yes	9 (75)	2 (40)	
No	3 (25)	3 (6)	
Total	12 (100)	5 (100)	0.4876
Use of HRT			
No	9 (69.23)	5 (71.43)	
Yes	4 (30.77)	2 (28.57)	
Total	13 (100)	7 (100)	1.0000
HBC			
Never used	18 (33.33)	6 (60)	
Had already used	9 (66.67)	4 (40)	
Total	27 (100)	10 (100)	0.7158
Family history of breast and ovarian cancer			
No	36 (72)	18 (72)	
Yes	14 (28)	7 (28)	
Total	50 (100)	25 (100)	1.0000
Alcoholism			
No	36 (92.31)	20 (100)	
Yes	3 (7.69)	0 (0)	
Total	39 (100)	20 (100)	0.5441
Smoking			
Never smoked	41 (77.36)	21 (70)	
Had already smoked	12 (22.64)	9 (30)	
Total	53 (100)	30 (100)	0.5999
Comorbidities			
Without comorbidities	11 (20.37)	13 (44.83)	
HBP	17 (31.48)	9 (31.03)	
DM	8 (14.81)	3 (10.34)	
Dyslipidemia	7 (12.96)	2 (6.90)	
Hypothyroidism	5 (9.26)	2 (6.90)	
Osteoporosis	6 (11.11)	0 (0)	
Total	54 (100)	29 (100)	0.1470
BMI			
Median (IQR) (kg/m^2)	25 (23-29)	27 (23-30.5)	
Total	51 (100)	28 (100)	0.7118
Clinical staging			
0	0 (0)	0 (0)	
I	7 (11.11)	0 (0)	
IIA	17 (26.98)	7 (21.88)	
IIB	16 (25.40)	5 (15.62)	
IIIA	9 (14.29)	5 (15.62)	
IIIB	12 (19.05)	13 (40.63)	
IIIC	1 (1.59)	0 (0)	
IV	1 (1.59)	2 (6.25)	
Total	63 (100)	32 (100)	0.1073
Relapse			
Yes	13 (36.11)	22 (73.33)	
No	23 (63.89)	8 (26.67)	
Total	36 (100)	30 (100)	0.0032

**The percentages may be greater than 100%, since patients may have more than one complaining for appointment.*
SD: standard deviation; IQR: interquartile range; HRT: hormone replacement therapy; HBC: hormonal birth control; HBP: high blood pressure; DM: diabetes mellitus*; BMI: body mass index.*

Histomorphological features

The histological types of TNBC in living women and patients who died were invasive ductal not otherwise specified (NOS) (93.75% vs. 93.94%), with apocrine differentiation (1.56% vs. 6.06%), metaplastic of no special type (3.13% vs. 0%) and medullary (1.56% vs. 0%) ($p = 0.3960$).

The angiolymphatic invasion was present in 48.44% of living women, and in 87.88% of those who died ($p = 0.0001$).

The nuclear pleomorphism in both groups were intermediate grade (3.13% vs. 9.09%) and high grade (96.88% vs. 90.91%) ($p = 0.2080$). The tubular differentiation in living patients and

in those who died were between 0% and 75% (43.75% vs. 33.33%) and < 10% (56.25% vs. 66.67%) (p = 0.3215). The mitotic index in living patients and those who died was ≤ 3 mitosis/mm^2 (45.31% vs. 39.39%), 4-7 mitosis/mm^2 (27.50% vs. 39.39%) and ≥ 8 mitosis/mm^2 (17.19% vs. 21.21%) (p = 0.8240). The histological grade of living patients who died and was I (1.56% vs. 3.03%), II (53.13% vs. 51.52%) and III (45.31% vs. 45.45%) (p = 0.8880).

The stages of the most common primary tumor for both patients were pT1c (22.58% vs. 10%), pT2 (54.84% vs. 46.67%), pT3 (11.29% vs. 10%), pT4b (3.23% vs. 16.67%) and pT4d (1.61% vs. 13.33%) (p = 0.0257). The most frequently stages of lymph node invasion for living women and for those who died were pN0 (61.29% vs. 30%), pN1a (25.81% vs. 30%), pN2a (11.29% vs. 20%), pN3a (0% vs. 20%) (p = 0.0012).

Treatment

The most frequently types of surgical treatments for living patients and to those who died were, modified radical mastectomy (64.52% vs. 73.33%), breast-conserving surgery (33.87% vs. 6.67%) and Halsted radical mastectomy (0% vs. 16.67%) (p = 0.0010). In both groups, the most common forms of radiotherapy were only adjuvant radiotherapy (69.35% vs. 56.25%) and only palliative radiotherapy (4.84% vs. 18.75%) and did not receive radiotherapy (25.81% vs. 21.87%) (p = 0.0750). In living patients and those who died, the modalities of chemotherapy were neoadjuvant chemotherapy (8.06% vs. 6.25%), adjuvant chemotherapy (67.74% vs. 21.88%), palliative chemotherapy (3.23% vs. 9.37%), neoadjuvant and adjuvant chemotherapy (12.90% vs. 6.25%), neoadjuvant, adjuvant and palliative chemotherapy (1.61% vs. 12.50%), neoadjuvant and palliative chemotherapy (1.61% vs. 12.50%), adjuvant and palliative chemotherapy (0% vs. 28.13%) and did not receive chemotherapy (for refusal, contraindication due to poor general condition, low rate of benefit, and unknown) (4.84% vs. 3.13%) (p < 0.0001).

Significant variables

Data are presented in **Table 5**.

Cox regression

In Cox regression, the variables angiolymphatic invasion, tumor size (pT), lymph node invasion (pN) and the type of surgery were used, in addition to follow-up and the outcome (alive and deaths). The angiolymphatic invasion obtained p = 0.0121 (relative risk [RR] 5.0518, confidence interval [CI] 95%

TABLE 5 – Significant clinicopathological and treatment variables*

Variables	Living *n* (%)	Deaths *n* (%)	*p* value
Time of follow-up			
Median (IQR) (months)	36.5 (22.5-77)	27 (14.8-40.8)	
Total	64 (100)	33 (100)	0.0401
Relapse			
Yes	13 (36.11)	22 (73.33)	
No	23 (63.89)	8 (26.67)	
Total	36 (100)	30 (100)	0.0032
Angiolymphatic invasion			
Present	31 (48.44)	29 (87.88)	
Absent	33 (51.56)	4 (12.12)	
Total	64 (100)	64 (100)	0.0001
Pathological staging			
Primary tumor (size)			
pTX	0 (0)	1 (3.33)	
pT0	0 (0)	0 (0)	
pTis	0 (0)	0 (0)	
pT1mi	0 (0)	0 (0)	
pT1a	1 (1.61)	0 (0)	
pT1b	3 (4.84)	0 (0)	
pT1c	14 (22.58)	3 (10)	
pT2	34 (54.84)	14 (46.67)	
pT3	7 (11.29)	3 (10)	
pT4a	0 (0)	0 (0)	
pT4b	2 (3.23)	5 (16.67)	
pT4c	0 (0)	0 (0)	
pT4d	1 (1.61)	4 (13.33)	
Total	62 (100)	30 (100)	0.0257
Lymph node invasion			
pNX	1 (1.61)	0 (0)	
pN0	38 (61.29)	9 (30)	
pN1mi	0 (0)	0 (0)	
pN1a	16 (25.81)	9 (30)	
pN2a	7 (11.29)	6 (20)	
pN3a	0 (0)	6 (20)	
Total	62 (100)	30 (100)	0.0012
Surgery			
Modified radical mastectomy	40 (64.52)	22 (73.33)	
Breast-conserving surgery	21 (33.87)	2 (6.67)	
Halsted radical mastectomy	0 (0)	5 (16.67)	
Total or simple mastectomy	1 (1.61)	1 (3.33)	
Total	62 (100)	30 (100)	0.001
Chemotherapy			
Neoadjuvant chemotherapy	5 (8.06)	2 (6.25)	
Adjuvant chemotherapy	42 (67.74)	7 (21.88)	
Palliative chemotherapy	2 (3.23)	3 (9.37)	
Neoadjuvant and adjuvant chemotherapy	8 (12.90)	2 (6.25)	
Neoadjuvant, adjuvant and palliative chemotherapy	1 (1.61)	4 (12.50)	
Neoadjuvant and palliative chemotherapy	1 (1.61)	4 (12.50)	
Adjuvant and palliative chemotherapy	0 (0.00)	9 (28.13)	
Did not receive chemotherapy	3 (4.84)	1 (3.13)	
Total	62 (100)	32 (100)	< 0.0001

** The other variables were not significant in the univariate analysis between groups. IQR: interquartile range.*

1.4261-17.8952) and tumor size, $p = 0.0385$ (RR 1.2605, CI 95% 1.0123-1.5695).

DISCUSSION

TNBC is a molecular subtype characterized by affecting younger women and for presenting unfavorable histopathological features, including high histological grade, high mitotic count, high risk of recurrence, lower and global survival free of disease[4]. It is considered one of the phenotypes of worst prognosis. However there are few studies in the literature defining the characteristics between patients with TNBC, which lead to have or not to have a worst prognosis.

Despite the age at TNBC diagnosis is related to women younger than 50 years, in our study the average age was 54.80 (± 14.10), similar to not TN tumors[2]. In addition, there was no statistical difference between the groups, although Shen *et al*. indicate the age as an independent prognostic factor[9].

Regarding the symptoms that lead the patient to the doctor, there was no statistical significance between living patients and those who died. However, the literature shows that with regard to receptor positive tumors, TNBC is less likely to be diagnosed by abnormal routine mammography. More than two thirds of patients with this subtype have symptoms, most commonly a palpable mass[10]. Our data reflect such outcome, since 64% of patients sought care because of a lump in the breast and only 16% due to changes in routine mammogram.

Lin *et al*. reported that menarche and first early pregnancy, greater parity, and shorter duration of breastfeeding are associated with TNBC[10]. In our study, early age at menarche (≤ 12 years)[11] did not show to act as a risk factor for development and mortality, from all causes, of TNBC. Our sample confirmed the literature data on early gestational age (< 25 years)[12], since the average found in the total sample was 21.87 (± 3.44) years, however there was no statistical difference between the groups. Regarding higher parity (≤ 3 pregnancies)[12], our work corroborated the published data, since the median was 3 pregnancies for all groups, but there was no statistical difference between them. According to research, women who breastfed for more than four months had reduced risk for TNBC[13, 14]. In our study, the median breastfeeding was nine months; about a third of patients did not breastfeed. In the latter, the absence of breastfeeding may have acted as a risk factor for the development of BC. Although median breastfeeding time was lower among the patients who died, there was no statistical significance.

According to Islam *et al*., menopause in women older than 50 years acts as a risk factor for BC in general, including TNBC[11]. Our study, as well as other studies, showed no association between late menopause and development of TNBC and a worse outcome[15].

The studies showed no association between the risk of TNBC and the use of HBC/C or HRT[11, 12, 16]. In our research, there was not statistically significant on the use of such therapies between the groups.

Kawai *et al*. did not associate smoking with TNBC[17]. In our analysis, most patients reported never smoking, so that we did not evidence statistical difference between living women and the patients who died.

Alcohol consumption has been associated with risk of BC in general. However Kabat *et al*. demonstrated that the risk of developing TNBC was lower among consumers of alcohol[18]. In our research, despite the alcoholic women belong to living women, they represented only 5.08%, with no statistical difference between the groups.

Most evidence suggests that the risk of BC with positive receptors is higher in obese postmenopausal women, while in obese premenopausal women is the risk of TNBC is greater[19]. In our analysis, BMI kept an overweight median, with no statistical significance.

Haffty *et al*. reported high proportion of family history of BC with development of TNBC[20]. We find this relationship in 28% of our patients, confirming the literature data. However, in our sample, this variable did not influence prognosis.

The most common comorbidities among the patients in our study were hypertension (HBP), diabetes mellitus (DM), and dyslipidemia. There was no statistical difference between the groups, and we did not find published data on the influence of comorbidities on the outcome of TNBC.

Yuan *et al*. suggest that high clinical stage leads to a worse prognosis[2]. The stage III corresponded to 56.25% of our patients who died, against 34.93% of those who remained alive, however there was no statistical significance.

TNBC has as one of its main characteristics the highest recurrence[4]. Our research showed recurrence rate of 53%, regardless of location, higher than the literature data[2], and was significantly related to the patients who died ($p = 0.0032$). This result puts the recurrence as an important risk factor for death, from all causes, in patients with TNBC. Also, Yuan *et al*. suggest that the recurrence reaches its maximum in the second year after diagnosis, decreasing thereafter[2], and that the metastasis

and death rates of TNBC increase precisely in this period (15 to 25 months of follow-up)[3, 10]. In our study, there was significant difference related to the time of follow-up until the outcome ($p = 0.0401$), and in patients who died, the median was 27 months, which confirms the literature data.

According to Elnashar *et al*., the majority of TNBC cases are invasive ductal NOS, medullary and metaplastic[21]. Our research is in line with the literature by presenting such histological types and demonstrate the high prevalence of ductal type[2]. There was no statistical difference between the groups.

The angiolymphatic invasion in our study was statistically significant among living patients and those who died ($p = 0.0001$). This data complies with published data, which claim that this is an important prognostic variable[2].

Although the TNBC is associated with a high mitotic index[4], in our analysis this has not occurred, because 43.30% of patients had an index of ≤ 3 mitosis/mm^2. However, in patients who died, a higher mitotic index was observed, but there was no statistical significance. Obeying literature, our sample had high nuclear pleomorphism index, but no statistical difference between the groups. Zhang *et al*. show that most patients with TNBC shows histological grade III[2, 4], which is a factor that decreases the survival time free of disease[3]. In our research, however, most patients remained in histological grade II, showing no significant difference.

Regarding the primary tumor, the diameter of TNBC is most commonly between 2 cm and 5 cm[2]; confirming the published data, most of our patients proved to be pT2. There was statistical difference between groups ($p = 0.0257$), since the patients who died had greater tumor size. Lymph node invasion showed statistical difference between groups ($p = 0.0012$), which is also described in the literature[2]. The majority (61.29%) of living patients was pN0, while 70% of patients who died had one or more lymph nodes metastases, and 20% of them had more than 10 lymph nodes.

Most of our patients underwent modified radical mastectomy (MRM) (64.89%), with statistically significant differences between groups ($p = 0.0010$). The prevalence of MRM in both groups complies with the literature[3]. Women who remained alive underwent conservative surgery in greater proportion compared with the other group. Gangi *et al*. demonstrated that breast-conserving surgery in TN phenotype is not related with the increase of local recurrence, and is not, therefore, a contraindicated strategy for this type of neoplasia, as was reported by some researchers[22].

Radiotherapy is indicated for most patients who undergo conservative therapy of the breast and also for a subgroup of patients with high-risk features for locoregional recurrence (a number of positive lymph nodes, tumor > 5 cm, angiolymphatic invasion or positive surgical margins). The Steward *et al*. study on the impact of adjuvant radiation in patients with TNBC indicated increased overall survival in patients undergoing breast conserving therapy and radiation, which was not evident in patients undergoing mastectomy[23]. In our research, 75.53% of patients underwent radiotherapy, and the adjuvant modality the most common in both groups, with no statistical difference. Currently, there are no specific guidelines for the management of TNBC[23], therefore further studies are needed to assist in the choice of whether using or not the radiation.

Chemotherapy is the only systemic therapy available for TNBC, and is the treatment base. Joensuu *et al*. suggest that chemotherapy is associated with increased overall survival and free of recurrence in patients with TNBC[24]. In our study, a statistically significant difference between groups ($p < 0.0001$) and, among the patients who died, most held adjuvant and palliative chemotherapy. Regarding neoadjuvant, TNBC has a favorable response in rates higher than 20% with this type of therapy. However, if there is no evidence of tumor regression, the chemotherapy regimen must change or perform surgery to not miss the opportunity to make treatment potentially effective[25]. Responses to neoadjuvant and adjuvant chemotherapy are important independent prognostic factors[21, 26]. According to Schmadeka *et al*. study, published in 2014, with the advancement of techniques of genetic and molecular profiling analyzes, new therapeutic targets for TNBC are being discovered. Biomarkers and pathways involved in the oncogenesis of TNBC are being explored to produce information and evaluate possible therapeutic intervention methods, which may favorably change the prognosis of this type of neoplasm[27].

The analysis by Cox regression revealed that tumor size and angiolymphatic invasion act as independent factors for prognosis. This result appears to be consistent with published data which show the same factors as the main prognostic indicators for TNBC[28, 29]. The literature adds other factors such as age, treatment and especially lymph node invasion[30], but in our study the lymph node invasion was significant only in the univariate analysis. In addition, studies indicate that chemotherapy and surgery influence on disease-free survival, and these variables were statistically significant in our survey[30, 31].

CONCLUSION

BC is a heterogeneous disease with various molecular subtypes that determine their behavior. The TN phenotype presents one of the worst prognoses, showing aggressive biological characteristics. Our analysis demonstrated the clinical and histopathological aspects of female patients with TNBC in the region of the Campos Gerais do Paraná, determines the profile of treatment and suggested that variables may predict poor prognosis. The angiolymphatic invasion and tumor size were shown to be risk factors for death, from all cases, in patients with TNBC. Additionally, such differences between groups may indicate different molecular subtypes among the TN phenotype. Thus, this study adds information to the literature that can help to define prevention methods, as well as improve the detection and assist in the development, planning and treatment to be spent in this terrifying type of BC.

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RESUMO

Introdução: *O câncer de mama é a neoplasia maligna visceral mais frequente em mulheres, sendo a principal causa de morte por câncer no sexo feminino em todo o mundo. O subgrupo triplo negativo apresenta prognóstico pobre e comportamento biológico agressivo.* ***Objetivos:*** *Delinear os aspectos clínicos e histomorfológicos e o perfil de tratamento, além de sugerir quais fatores podem predizer pior prognóstico nas pacientes com câncer de mama invasivo triplo negativo na região dos Campos Gerais do Paraná.* ***Métodos:*** *Estudo de corte retrospectivo observacional, longitudinal e comparativo, realizado em uma clínica de anatomia patológica e no Instituto Sul Paranaense de Oncologia, em Ponta Grossa, Paraná. Os critérios de inclusão foram pacientes do sexo feminino com laudo anatomopatológico de carcinoma de mama invasor, cuja imuno-histoquímica apresentou-se negativa para os receptores hormonais e receptor de crescimento epidérmico humano 2 (HER2), diagnosticadas no período entre 01 de janeiro de 2002 a 31 de dezembro de 2012. As pacientes foram divididas em dois grupos, mulheres vivas e as que faleceram.* ***Resultados:*** *O índice de recidiva, o tipo de quimioterapia, a invasão angiolinfática, o tamanho do tumor, a invasão linfonodal e os tipos de cirurgia realizadas foram variáveis significativas na análise univariada entre os grupos. Após a regressão de cox para análise multivariada, apenas a invasão angiolinfática (*p = *0,012, risco relativo [RR] 5,0518, intervalo de confiança [IC] 95% 1,4261-17,8952) e o tamanho do tumor permaneceram significativos (*p = *0,0385, RR 1,2605, IC 95% 1,0123-1,5695).* ***Conclusão:*** *A invasão angiolinfática e o tamanho do tumor mostraram-se fatores de risco para óbito, por todas as causas, em pacientes com câncer de mama triplo negativo. Diferenças entre os grupos podem indicar diferentes subtipos moleculares dentro do fenótipo triplo negativo.*

Unitermos: *neoplasias da mama; imuno-histoquímica; prognóstico; óbito.*

REFERENCES

1. INCA. Instituto Nacional de Câncer [internet]. Available at: http://www.inca.gov.br/estimativa/2014/sintese-de-resultados-comentarios.asp. [Acesso em: 18 abril 2014].

2. Yuan N, Meng M, Liu C, et al. Clinical characteristics and prognostic analysis of triple-negative breast cancer patients. Mol Clin Oncol. 2014; 2(2): 245-51. PubMed PMID: 24649341.

3. Zhang L, Hao C, Dong G, Tong Z. Analysis of clinical features and outcome of 356 triple-negative breast cancer patients in China. Breast Care (Basel). 2012; 7(1): 13-7. PubMed PMID: 22553467.

4. Konigsberg R, Pfeiler G, Klement T, et al. Tumor characteristics and recurrence patterns in triple negative breast cancer: a comparison between younger (< 65) and elderly (≥ 65) patients. Eur J Cancer. 2012; 48(16): 2962-8. PubMed PMID: 22647688.

5. Giraldo-Jiménez MY, Cabanillas F, Negrón V, et al. Triple negative breast cancer: a retrospective study of Hispanics residing in Puerto Rico. P R Helth Sci J. 2012; 31(2): 45-51. PubMed PMID: 22783695.

6. Fitzgibbons PL, Dillon DA, Alsabeh R, et al. Template for reporting results of biomarker testing of specimens from patients with carcinoma of the breast. Arch Pathol Lab Med. 2014; 138(5):595-601. PubMed PMID: 24236805.

7. Sobin L, Gospodarowicz M, Wittekind C. Classification of malignant tumors. 7th ed. UICC Internation Union Against Cancer; 2010.

8. College of American Pathologists. Protocol of the examination of specimens from patients with invasive carcinoma of the breast. Invasive Breast 3.2.0.0; 2013.

9. Shen SJ, Sun Q, Zhou YD, et al. Analysisof prognostic factors related to triple-negative breast cancer. Zhonghua Wai Ke Za Zhi. 2013; 51(11): 1000-4. PubMed PMID: 24444685.

10. Lin N, Vanderplas A, Hughes M, et al. Clinicopathological features, patterns of recurrence, and survival among women with triple-negative breast cancer in the national comprehensive cancer network. Cancer. 2012; 118(22): 5463-72. PubMed PMID: 22544643.

11. Islam T, Matsuo K, Ito H, Hosono S, Watanabe M, Iwata H. Reproductive and hormonal risk factors for luminal, Her2-overexpressing, and triple-negative breast cancer in Japanese women. Ann Oncol. 2012; 23(9): 2435-41. PubMed PMID: 22328736.

12. Martinez ME, Wertheim BC, Natarajan L, et al. Reproductive factors, heterogeneity, and breast tumor subtypes in women of mexican descent. Cancer Epidemiol Biomarkers Prev. 2013; 22(10): 1853-61. PubMed PMID: 23950213.

13. Phipps AI, Malone KE, Porter PL, Daling JR, Li CI. Reproductive and hormonal risk factors for postmenopausal luminal, HER2-overexpressing, and triple-negative breast cancer. Cancer. 2008; 113(7): 1521-26. PubMed PMID: 18726992.

14. Phipps AI, Chlebowski RT, Prentice R, et al. Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. J Natl Cancer Inst. 2011; 103(6): 470-7. PubMed PMID: 21346227.

15. Anderson KN, Schwab RB, Martinez ME. Reproductive risk factors and breast cancer subtypes: a review of the literature. Breast Cancer Res Treat. 2014; 144(1): 1-10. PubMed PMID: 24477977.

16. Ambrosone CB, Zirpoli G, Ruszczyk M, et al. Parity and breastfeeding among African-American women: differential effects on breast cancer risk by estrogen receptor status in the Women's Circle of Health Study. Cancer Causes Control. 2014; 25(2): 259-65. PubMed PMID: 24249438.

17. Kawai M, Malone KE, Tang MT, Li CI. Active smoking and the risk of estrogen receptor-positive and triple-negative breast cancer among women ages 20 to 44 years. Cancer. 2014; 120(7): 1026-34. PubMed PMID: 24515648.

18. Kabat GC, Kim M, Phipps AI, et al. Smoking and alcohol consumption in relation to risk of triple-negative breast cancer in a cohort postmenopausal women. Cancer Causes Control. 2011; 22(5): 775-83. PubMed PMID: 21360045.

19. Jain R, Strickler HD, Fine E, Sparano JA. Clinical studies examining the impact of obesity on breast cancer risk and prognosis. J Mammary Gland Biol Neoplasia. 2013; 18(3-4): 257-66. PubMed PMID: 24221746.

20. Haffty BG, Yang Q, Reiss M, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol. 2006; 24: 5652-7. PubMed PMID: 17116942.

21. Elnashar AT, Ali el-SM, Gaber A. The prognostic value of triple negative in stage II/III breast cancer. J Oncol Pharm Pract. 2012; 18(1): 68-75. PubMed PMID: 21422149.

22. Gangi A, Chung A, Mirocha J, Liou DZ, Leong T, Giuliano AE. Breast-conserving therapy for triple-negative breast cancer. JAMA Surg. 2014; 149(3): 252-8. PubMed PMID: 24382582.

23. Steward LT, Gao F, Taylor MA, Margenthaler JA. Impact of radiation therapy on survival in patients with triple-negative breast cancer. Oncol Lett. 2014; 7(2): 548-52. PubMed PMID: 24396485.

24. Joensuu H, Gligorov J. Adjuvant treatments for triple-negative breast cancers. Ann Oncol. 2012; 23(6): 40-5. PubMed PMID: 23012301.

25. Yagata H, Kajiura Y, Yamauchi H. Current strategy for triple-negative breast cancer: appropriate combination of surgery, radiation, and chemotherapy. Breast Cancer. 2011; 18(3): 165-73. PubMed PMID: 21290263.

26. Asaga S, Kinoshita T, Hojo T, Suzuki J, Jimbo K, Tsuda H. Prognostic factors for triple-negative breast cancer patients receiving preoperative systemic chemotherapy. Clin Breast Cancer. 2013; 13(1): 40-6. PubMed PMID: 23103363.

27. Schmadeka R, Harmon BE, Singh M. Triple negative breast carcinoma: current and emerging concepts. Am J Clin Pathol. 2014; 141(4): 462-77. PudMed PMID: 24619745.

28. Pistelli M, Pagliacci A, Battelli N, et al. Prognostic factors in early-stage triple-negative breast cancer: lessons and limits from clinical practice. Anticancer Res. 2013; 33(6): 2737-42. PubMed PMID: 23749934.

29. Yao-Lung K, Dar-Ren C, Tsai-Wang C. Clinicopathological features of triple-negative breast cancer in Taiwanese women. Int J Clin Oncol. 2011; 16(5): 500-5. PubMed PMID: 21455625.

30. Shen SJ, Sun Q, Zhou YD, et al. Analysis of prognostic factors related to triple-negative breast cancer. Zhonghua Wai Ke Za Zhi. 2013; 51(11): 1000-4. PubMed PMID: 24444685.

31. Li CY, Wang P, Zhang S, Liu Y, Zhang J. Clinicopathological features and prognosis of triple-negative breast cancer. Zhonghua Zhong Liu Za Zhi. 2013; 35(6): 463-7. PubMed PMID: 24119909.

MAILING ADDRESS

Camila M. Lopes
Rua Estados Unidos, 291, apto 102; Centro; CEP: 84010-610; Ponta Grossa-PR, Brazil; Phone: +55 (42) 9929-8255; e-mail: camila1992lopes@gmail.com.