British Journal of Medicine & Medical Research
3(4): 880-896, 2013

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Triple-Negative Breast Cancer in Brazilian Women without Metastasis to Axillary Lymph Nodes: Ten-Year Survival and Prognostic Factors

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Authors' contributions

This work was carried out in collaboration between all authors. All authors contributed to the interpretation of the data and were responsible for reviewing the manuscript. All authors have read and approved the final manuscript.

Research Article

Received 9th November 2012
Accepted 9th February 2013
Published 11th March 2013

ABSTRACT

Aims: To determine the 10-year overall survival (OS) in triple-negative (TN) and non-TN breast cancer (BC) patients, and to identify associated independent prognostic factors.
Study Design: Descriptive and survival.
Place and Duration of Study: Pathology Division at National Cancer Institute, Rio de Janeiro, Brazil, between 1992-1996.
Methodology: Population: 348 women patients with invasive ductal carcinoma without lymph node metastasis. Analyzed variables: age, treatment, surgery type, tumor size, skin involvement, histological grade, vascular invasion, estrogen and progesterone receptors, HER-2, Ki-67 and p53. Statistical analysis performed: Kaplan-Meier survival curves, log rank test, and multivariate Cox models.
Results: 27% of the studied women were categorized as TNBC and 73%, as non-TNBC. The former showed higher frequency of age <50yr, preoperative chemotherapy, tumors

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>5cm, high grade, vascular invasion, and positive p53, (*P*=.05). Ten-year OS among TNBC patients was 61.6%, and 70.1% for non-TNBC patients (*P*=.058). Survival was higher in TNBC patients treated with partial surgeries, tumors ≤5cm, without skin involvement, low grade, and Ki-67 negative (*P*=.05). Among non-TNBC patients, higher survival was observed in patients without skin involvement, low grade, no vascular invasion, and p53 negative, (*P*=.05). Cox modelization showed a 2-fold higher death risk for TNBC patients aged ≥50yr, about 2.5-fold higher risk related to preoperative chemotherapy, high grade tumor and skin involvement, and a 3.0-fold higher risk for Ki-67 positive patients (*P*=.05). For non-TNBC patients, a 2.0-fold increased death risk was verified in patients with skin involvement and vascular invasion (*P*=.05).

Conclusion: TNBC patients showed a worse prognosis and survival when compared to non-TNBC patients. A worse 10-yr survival among TNBC patients was associated with age ≥50yr, preoperative chemotherapy, skin involvement, high histological grade, and Ki-67 positive tumors. For non-TNBC patients, the worst prognosis was related to skin involvement and vascular invasion. These predictors need to be further validating by other studies.

Keywords: Breast cancer; triple-negative breast cancer; survival; prognostic factors.

ABBREVIATIONS

BC: breast cancer; CI: confidence interval; DAB: diaminobenzidine; ER: estrogen; receptor; FDA: Food and Drug Administration; H2O2: hydrogen peroxide solution; HER-2: Human epidermal growth factor receptor type 2; HR: hazard ratios; IDC: invasive ductal carcinoma; IDC-NST: invasive ductal carcinoma of no special type; INCA: National Institute of Cancer; NPI: Nottingham prognostic index; non-TN: non triple-negative; OS: Overall survival; PAP: peroxidase-antiperoxidase; PR: progesterone receptor; sd: standard deviation; SIM: Mortality Information System; SUS: National Health System of Brazil; TBS: tris buffer solution; TN: triple-negative.

1. INTRODUCTION

Breast cancer (BC) is a heterogeneous disease that encompasses a variety of entities with distinct clinical behavior and morphological aspects. Recently, it has become more evident that this diversity results from distinct genetic alterations [1-6]. Although morphology is frequently associated with patterns of molecular aberrations, it is also clear that tumors of the same histological type show remarkable different clinical behavior [5]. At least, five molecular BC subtypes have already been described: luminal A, luminal B, normal breast-like, HER-2/cerbB-2 positive, and basal-like tumors [5,7-9].

On immunohistochemical analysis, triple-negative BC is a heterogenous group of any histological type of BC that has decreased or lacks expression of estrogen receptor (ER), progesterone receptor (PR) and HER2, thus characterizing a triple-negative immunophenotype [5,6,10,11]. The terms “triple-negative” and “basal-like” are often used interchangeably, because both subtypes often overlap. Both tumors are more prevalent in young women (< 50 years), and the major histological type is invasive ductal carcinoma (IDC) of no special type (IDC-NST), with high histological grade (grade 3) and associated with a very poor prognosis [4,5,11-15].

Triple-negative BC currently poses a great clinical challenge, since there is still no specific and universally accepted targeted therapy for these tumors [10,11,13]. About 80 to 90% of triple-negative BC are basal-like tumors [8,12,16] and 56% to 90% of basal-like tumors are triple-negative BC [12,13,17]. Moreover, only 8 to 29% of triple-negative are not basal-like, and around 18 to 40% of basal-like tumors are not triple-negative [18,19].

The aim of this study was to determine the 10-year survival rate in patients with breast invasive ductal carcinoma (IDC) without metastasis to axillary lymph nodes, according to the presence/absence of immunohistochemical diagnosis of triple-negative neoplasm, as well as to identify the associated prognostic factors.

2. MATERIALS AND METHODS

This is a survival study of a hospital-based cohort of BC patients, diagnosed from January 1992 to December 1996 in the Brazilian National Institute of Cancer (INCA), an oncologic referral center providing universal and free care at the National Health System (SUS), located in the city of Rio de Janeiro.

A review of medical charts and/or histopathological reports was carried out. The following clinical variables were analyzed: patient age; date of histopathological diagnosis; modality of cancer treatment received, including surgery, radiation therapy, chemotherapy and hormone therapy; type of surgery performed (partial surgery/segmentectomy or radical surgery/mastectomy) and tumor size.

Starting from the date of disease diagnosis, patients were followed for ten years. The occurrence of death was categorized as failure. Censoring occurred when there was loss of patient follow-up or non-occurrence of death after the 120th month since diagnosis. Follow-up data was obtained from patient medical charts, nominal search in the mortality database of the National Mortality System (SIM) relative to the population residing in the State of Rio de Janeiro (available in electronic media until the year 2008), and active search by means of telephone contact, letters, and telegrams.

Slides containing tumor tissue and stained with hematoxylin-eosin were reviewed by the same pathologist (ALAE) who histologically classified lesions, according to the World Health Organization criteria [20]. Only IDC cases were included in the study. Skin involvement, histological grade [based on the Patey & Scarff [21] classification system and Bloom & Richardson [22], modified by Elston & Ellis [23], and vascular invasion were also evaluated. In all cases studied, estrogen receptor (ER), progesterone receptor (PR), HER-2, Ki67/MIB-1 and p53 were evaluated by immunohistochemistry.

Immunohistochemistry analysis was performed on formalin-fixed, paraffin-embedded breast cancer tissue, as previously described [8,9,13,24,25]. Positive and negative controls for each marker were used.

The specific antibodies used were antiestrogen monoclonal antibody (clone 1D5; 1/400; Dako) for ER, antiprogesterone monoclonal antibody (clone PGR2; 1/300; Novocastra) for PR and polyclonal antibody (anti-c*erb*B-2 oncoprotein; 1/1.600; Dako) for HER2.

For ER and PR, reactions were considered positive in tissues with at least 10% stained nuclei, and negative when there were less than 10% stained nuclei or no staining [9,13,25,26]. For the HER2 oncogene, a scoring system approved by the United States FDA

(Food and Drug Administration) was used for HercepTest® and consisted of three scores: 0 or 1+ = negative; 2+ and 3+ = positive [27,28]. Only membrane staining was considered; immunoreactivity was defined as positive (2+ or 3+) when more than 10% of tumor cells exhibited weak, moderate or intense and complete staining of the membrane; in the absence of staining or in incomplete staining or when staining was present in less than 10% of tumor cells, the reaction was considered negative (0/1+). For the present study, patients were classified as having triple-negative IDC, when ER, PR and HER2 were negative. These patients were considered non-triple-negative IDC when they tested positive for at least one of these markers. After stratification of the original cohort, 95 patients (27%) were classified as having triple-negative IDC and 253 (73%) as having non-triple-negative IDC.

Expression of Ki-67 (anti-Ki-67 monoclonal antibody; clone MIB-1; 1/200; Dako) and p53 (anti-p53 monoclonal antibody; clone DO-7; 1/200; Dako) were also evaluated. The evaluation of Ki-67 positivity was estimated (visual assessment) and a cutoff point of 20% was used to classify tumors into two strata: low proliferation index, when there were 20% or less stained nuclei (reaction considered negative) and a high proliferation index, when there were more than 20% of stained nuclei (positive reaction) [29, 30]. For p53, a cutoff point of 10% was used; the reaction was considered positive when 10% or more cells had stained nuclei, and negative, when at least 10% of cells had stained nuclei or when there was no staining [31,32].

Initially, a bivariate analysis (Pearson's chi-square) was performed to determine the presence of an association between clinicopathological history and triple-negative IDC. The probability of its occurrence by chance was estimated by Pearson's chi-square test, and a *P*-value <.05 was reported as statistically significant (alternative hypothesis).

Overall survival (OS) was calculated by the Kaplan-Meier method, defined as the time interval from histopathological diagnosis until death or last patient follow-up. Final status of the patient at the last follow-up was termed "dead" or "alive." Overall survival curves were constructed to compare women in both groups (triple-negative IDC and non-triple-negative IDC). The respective curves were compared by the log-rank test.

Determination of prognostic factors for survival was performed by using Cox multivariate regression analysis. Using the "enter forward" method, regression models were developed for the same clinic-pathological characteristics, and hazard ratios (HR), with their respective 95% confidence intervals (CI), were obtained for clinic-pathological variables related to overall survival [33].

Ethical issues: This study was approved by the Research Ethics Committee of INCA, number 109/2011.

3. RESULTS

The cohort analyzed included 348 patients with histopathological diagnosis of IDC without metastasis to axillary lymph nodes, undergoing surgery with or without adjuvant treatment. Ninety five of these women (27%) were categorized as triple-negative IDC, and 253 patients (73%) as non-triple-negative IDC. The mean follow-up period in this group was 97 months (95%CI=93-101 months), and at the end of follow-up of 120 months, 165 (47,4%) were alive, 102 (29,3%) died, and 81 (23,3%) had incomplete follow-up (two patients were lost to follow-up before the first death at 7 months; 41 were lost to follow-up between 11 e 59 months; and 38 were lost to follow-up between 60 and 116 months (data not shown).

Triple-negative IDC patients ranged 28-87 years, and the mean age was 53 years, sd= 13.7 (42, 52, and 63 years, respectively for the 1st, 2nd and 3rd quartiles). Among non-triple-negative IDC, age distribution ranged 25-83 years, and the mean age was 57.8 years, sd= 2.8 (49, 59, and 69 years, respectively for the 1st, 2nd and 3rd quartiles). The distribution of triple negative IDC tumor size ranged from 1.5 to 13.0 cm, mean tumor size 4.5 cm, sd=.28, and 1st, 2nd and 3rd quartiles, respectively, 3.0, 4.0 and 6.0 cm. Among non-triple-negative tumors, tumor size ranged from 1.0 to 12.0 cm, mean tumor size 3.8 cm, sd=.12, and 1st, 2nd and 3rd quartiles, respectively, 2.5, 3.5 and 5.0 cm (data not shown).

Table 1 shows the results obtained by bivariate analysis. It can be observed that patients with triple-negative IDC, compared to those with non-triple-negative IDC, showed a higher frequency of patients with: (a) age under 50 years (45% versus 27%; *P*=.001); (b) treatment with preoperative chemotherapy (22% versus 6%; *P*<.001); (c) tumors larger than 5.0 cm (48% versus 23%; *P*<.001); (d) tumors with high histological grade (62% versus 21%; *P*<.001), (e) tumors exhibiting vascular invasion (45% versus 34%; *P*=.04), and (f) p53 positive tumor cells (37% versus 18%; *P*<.001).

Table 1. Patients characteristics and tumor parameters at diagnosis, triple-negative and non-triple-negative breast cancer, Rio de Janeiro, Brazil

Variables Stratus	TN IDC n (%)	Non-TN IDC n (%)	Pearson χ^2	*P*-value
Age of patients				
< 50 years	43 (45.3)	68 (26.9)	10.748	.001
≥ 50 years	52 (54.7)	185 (73.1)		
Treatment*				
Surgery with or without Rxt and/or ch*	74 (77.9)	239 (94.5)	20.967	<.001
Preoperative ch*	21 (22.1)	14 (5.5)		
Type of surgery				
Partial/segmentectomy	20 (21.1)	59 (23.3)	0.202	.05
Radical/mastectomy	75 (78.9)	194 (76.7)		
Tumor size				
≤ 5 cm	46 (51.7)	182 (77.4)	20.546	<.001
> 5 cm	43 (48.3)	53 (22.6)		
Skin involvement				
No	73 (76.8)	214 (84.9)	3.148	.07
Yes	22 (23.2)	38 (15.1)		
Histological grade				
Grade 1+ 2 (low grade)	36 (37.9)	199 (78.7)	52.333	<.001
Grade 3 (high grade)	59 (62.1)	54 (21.3)		
Vascular invasion				
No	52 (54.7)	168 (64.2)	4.043	.04
Yes	43 (45.3)	85 (33.6)		
Ki-67				
Negative	35 (36.8)	120 (47.4)	3.135	.07
Positive	60 (63.2)	133 (52.6)		
p53				
Negative	60 (63.2)	208 (82.2)	14.166	<.001
Positive	35 (36.8)	45 (17.8)		

** with or without hormone therapy; Rxt: radioterapy; ch: chemotherapy; n: number; (%): percents; χ2: chi-square.*

The 10-year overall survival for triple-negative IDC patients was 61.6% (sd=5.2) and 70.1% (sd=3.1) for non-triple-negative IDC patients (log rank=3.595; *P*=.058) (Table 2; Fig. 1). Their mean survival were, respectively, 89 months (95%CI=81-98) and 100 months (95%CI=96-105), (data not shown). Cumulative mortality at 36, 60, and 96 months was always higher in triple-negative IDC patients (58.8%, 82.4% and 97.1%, respectively) than in non-triple-negative IDC patients (38.2%, 64.7% and 88.2%, respectively) (Table 2).

Table 2. Overall survival and deaths (number and accumulated percent) at 12, 36, 60, 96 and 120 months, triple-negative and non-triple-negative breast cancer, Rio de Janeiro, Brazil

	TN IDC	Non-TN IDC	Log rank	*P*-value
Overall survival at 12 months (±sd)	97.8% (±1.5)	98.4% (±0.8)	-	-
Number of deaths at 12 months; accumulated percent (%)	2 (5.9%)	4 (5.9%)	-	-
Overall survival at 36 months (±sd)	78.1% (±4.3)	89.6% (±1.9)	-	-
Number of deaths at 36 months; accumulated percent (%)	20 (58.8%)	26 (38.2%)	-	-
Overall survival at 60 months (±sd)	69.0% (±4.9)	82.2% (±2.4)	6.311	.01
Number of deaths at 60 months; accumulated percent (%)	28 (82.4%)	44 (64.7%)	-	-
Overall survival at 96 months (±sd)	62.8% (±5.2)	74.6% (±2.9)	-	-
Number of deaths at 96 months; accumulated percent (%)	33 (97.1%)	60 (88.2%)	-	-
Overall survival at 120 months (±sd)	61.6% (±5.2)	70.1% (±3.1)	3.595	.058
Number of deaths at 120 months; accumulated percent (%)	34 (100%)	68 (100%)	-	-

sd: standard deviation; TN: triple-negative; non-TN: non-triple-negative; IDC: invasive ductal carcinoma.

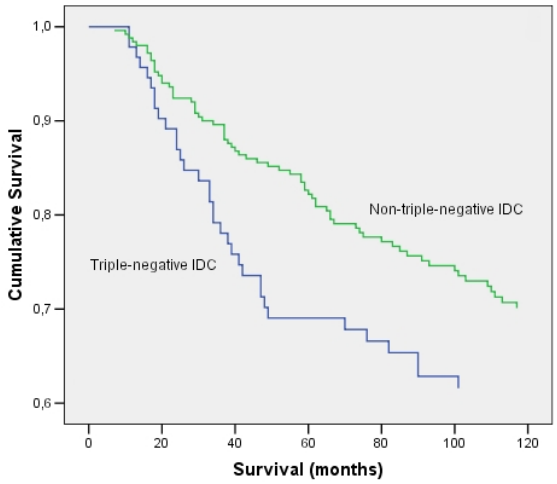

Fig. 1. Ten-year overall survival curves for patients with triple-negative (TN) and non-TN invasive ductal carcinoma (IDC) diagnosed between 1992-1996, Kaplan-Meier analysis (log-rank test, *P*-value=.058), Rio de Janeiro, Brazil

The results of Kaplan-Meier survival analysis for triple-negative and non-triple-negative patients are presented at Table 3. In triple-negative IDC, survival rates were higher in patients: (a) treated with surgery (with or without radiotherapy and/or chemotherapy, and with or without hormone therapy) compared to those treated with preoperative chemotherapy (with or without hormone therapy), respectively, 68.1% and 37.7%, *P*=.006; (b) undergoing partial surgeries/segmentectomies compared to those undergoing radical surgeries/ mastectomies, respectively, 88.9 % and 54.8%, *P*=.01; (c) with tumors measuring 5.0 cm or less compared to those with tumors measuring more than 5.0 cm, respectively, 76.7% and 45.2%, *P*=.004; (d) with tumors without skin involvement compared to those tumors with skin involvement, respectively, 71.6% and 30.7%, *P*<.001; (e) with low histological grade tumors (grades 1 + 2) compared to those with high histological grade tumors (grade 3), respectively, 78.1% and 52.3%, *P*=.02; and (f), with Ki-67 negative tumors compared to those with Ki-67 positive tumors, respectively, 77.6% and 53.1%, *P*=.02. For patients with non-triple-negative IDC, the variables associated with 10-year survival were: skin involvement (higher survival, when absent: 73.5% versus 51.9%, *P*=.005); histological grade [higher survival for lower histological grade tumors (grades 1 + 2): 73.2% versus 58.9%, *P*=.02]; vascular invasion (higher survival, when absent: 75.6% versus 59.0%, *P*<.001), and p53 (higher survival, when negative 73.0% versus 57.1%, *P*=.03).

On Cox multivariate analysis, empty models were initially tested. The likelihood ratios (-2 log likelihood) were 290.859 and 717.747 in models for triple-negative IDC and non-triple-negative IDC, respectively. Subsequently, variables that proved to be statistically significant in the Kaplan-Meier survival analysis were used in multivariate models to estimate their joint effect on survival of patients with triple-negative IDC and non-triple-negative IDC. Although just showing a non-statistically significance (*P*=.07) on survival (Kaplan-Meier analysis), age was tested in the Cox model for 95 patients with triple-negative IDC, considering its biological relevance to these tumors.

According to Cox model 1 obtained for patients with triple-negative IDC (Table 4), an estimated death risk two-fold higher in patients aged 50 years or older (HR=2.02, 95%CI =1.00-4.09), and a 2.75-fold increased death risk in patients with high histological grade tumors (grade 3), HR=2.75, 95%CI=1.19-6.35, were observed.

In the Cox model 2 ascertained for the same group of triple-negative IDC patients (Table 4), increased death risks were estimated for patients aged 50 years or more (HR=2.74, 95%CI=1.23-5.78), treated with preoperative chemotherapy (HR=2.41, 95%CI=1.08-5.37), with tumors with skin involvement (HR=2.80, 95%CI=1.33-5.88), and with Ki-67 positive tumors (HR=3.02, 95%CI=1.30-7.01).

In the Cox model obtained for patients with non-triple-negative IDC (Table 4), there was a two-fold increased risk of death in patients with tumors with skin involvement (HR=2.19, 95%CI=1.26-3.79), and in those with vascular invasion, (HR=2.20, 95%CI=1.37-3.54).

Table 3. Ten-year overall survival (Kaplan-Meier analysis) according clinicopathologic variables: triple-negative and non-triple-negative breast cancer, Rio de Janeiro, Brazil.

Variables / Stratus	TN IDC: total number of deaths (%)	TN IDC: 10-year overall survival (%) (±sd)	TN IDC: mean survival in months (95%CI); log rank test, *P*-value	Non-TN IDC: total number of deaths (%)	Non-TN IDC: 10-year overall survival (%) (±sd)	Non-TN IDC: mean survival in months (95%CI);); log rank test, *P*-value
Age			*P*=.07			*P*=.05
< 50 years	12 (35.3)	70.5 (±7.2)	98.3 (87.2-109.4)	18 (26.5)	72.3 (±5.6)	-
≥ 50 years	22 (64.7)	53.8 (±7.3)	81.4 (68.9-94.0)	50 (73.5)	69.1 (±3.7)	-
Treatment*			*P*=.006			
Surgery with/without Rxt	22 (64.7)	68.1 (±5.6)	95.4 (86.3-104.5)	64 (94.1)	70.0 (±3.2)	-
Preoperative ch*	12 (35.3)	37.7 (±11.1)	66.9 (47.1-86.7)	4 (5.9)	70.7 (±12.4)	-
Type of surgery			*P*=.01			
Partial/segmentectomy	2 (5.9)	88.9 (±7.4)	111.3 (99.9-122.7)	11 (16.2)	77.4 (±6.1)	-
Radical/mastectomy	32 (94.1)	54.8 (±6.0)	83.7 (73.6-93.7)	57 (83.8)	67.9 (±3.6)	-
Tumor size			*P*=.004			
≤ 5 cm	10 (31.2)	76.7 (±6.5)	101.8 (91.1-112.5)	50 (74.6)	69.1 (±3.7)	-
> 5 cm	22 (68.8)	45.2 (±7.9)	75.7 (62.3-89.2)	17 (25,4)	63.9 (±7.3)	-
Skin involvement			*P*=<.001			*P*=.005
No	19 (55.9)	71.6 (±5.5)	97.6 m (88.5-106.7)	51 (75)	73.5 (±3.2)	102.6 (98.1-107.1)
Yes	15 (44.1)	30.7 (±10.1)	63.3 (45.7-80.9)	17 (25)	51.9 (±8.6)	87.2 (73.3-101.0)
Histological grade			*P*=.02			*P*=.02
Grade 1+ 2 (low grade)	7 (20.6)	78.1 (±7.4)	103.2 (91.4-115.0)	48 (70.6)	73.2 (±3.4)	103.4 (98.8-108.0)
Grade 3 (high grade)	27 (79.4)	52.3 (±6.7)	81.4 (70.1-92.6)	20 (29.4)	58.9 (±7.3)	88.6 (77.3-99.9)
Vascular invasion						*P*=<.001
No	16 (47.1)	66.5 (±6.9)	-	35 (51.5)	75.6 (±3.7)	106.6 (102.1-111.1)
Yes	18 (52.9)	55.6 (±7.9)	-	33 (48.5)	59.0 (±5.5)	87.8 (78.8-96.8)
Ki-67			*P*=.02			
Negative	7 (20.6)	77.6 (±7.5)	103.7 (92.7-114.8)	27 (39.7)	74.4 (±4.3)	-
Positive	27 (79.4)	53.1 (±6.6)	81.5 (70.0-92.9)	41 (60.3)	66.1 (±4.4)	-
p53						*P*=.03
Negative	21 (61.8)	63.3 (±6.4)	-	50 (73.5)	73.0 (±3.3)	102.7 (98.1-107.3)
Positive	13 (38.2)	58.2 (±9.0)	-	18 (26.5)	57.1 (±7.9)	89.7 (77.7-101.6)

**with or without hormone therapy; **Rxt: radiotherapy and/or ch: chemotherapy; IDC: invasive ductal carcinoma; sd: standard deviation. Note: survival was not calculated for variables not statistically significant.*

Table 4. Independent prognostic factors associated at 10-year overall survival (Cox models), triple-negative and non-triple-negative breast cancer, Rio de Janeiro, Brazil.

Variables / Stratus	β	Statistic Wald	*P*-value	HR (95%CI)
Triple-negative IDC – Model 1				
Age				
< 50 years	0.70	3.78	.05	
≥ 50 years				2.02 (1.00-4.09)
Histological grade				
Grade 1 + 2 (low grade)	1.01	5.63	.02	
Grade 3 (high grade)				2.75 (1.19-6.35)
Triple-negative IDC – Model 2				
Age				
< 50 years	1.01	6.98	.008	
≥ 50 years				2.74 (1.23-5.78)
Treatment*				
Surgery with or without Rxt and/or ch*	0.88	4.63	.03	
Preoperative ch*				2.41 (1.08-5.37)
Skin involvement				
No	1.03	7.41	.006	
Yes				2.80 (1.33-5.88)
Ki-67				
Negative	1.11	6.60	.01	
Positive				3.02 (1.30-7.01)
Non-triple-negative IDC				
Skin involvement				
No	0.78	7.76	.005	
Yes				2.19 (1.26-3.79)
Vascular invasion				
No	0.79	10.54	.001	
Yes				2.20 (1.37-3.54)

*IDC: invasive ductal carcinoma; *with or without hormone therapy; Rxt: radiotherapy; ch: chemotherapy; β: beta value; p-value: statistic significance; Exp (β): beta exponential = HR: hazard ratio; 95%CI: 95% confidence interval; one degree of freedom for each variable.*

Values of first model for triple-negative IDC: -2 log likelihood = 281.050; $\chi 2$ = 9.809; degrees of freedom = 2; P-value = .007.

Values of second model for triple-negative IDC: -2 log likelihood = 264.747; $\chi 2$ = 26.821; degrees of freedom = 4; P-value < .001.

Values of the model for non triple-negative IDC: -2 log likelihood = 701.932; $\chi 2$ = 18.932; degrees of freedom = 2; P-value < .001.

4. DISCUSSION

In this study, the 10-year overall survival was lower in patients with triple-negative IDC (61.6%) than in those with non-triple-negative IDC (70.1%). The independent predictive factors of worse prognosis for 10-year survival in patients with triple-negative IDC were age 50 years or older, treatment with preoperative chemotherapy, skin involvement, higher histological grade (grade 3) and Ki-67 positive tumors, which presented two to three-fold increased risks of death. For patients with non-triple-negative IDC, the independent factors for worse prognosis were skin involvement and vascular invasion, with a two-fold increased

mortality risk. These findings reinforce evidence existing in the literature that BC is a heterogenenous disease. Furthermore, triple-negative BCs deserve special attention because their biological behavior is distinct than observed in other subtypes, besides showing a worse prognosis [7-9,34-36].

According to some authors, triple-negative and/or basal-like BC aggressiveness is higher at the disease onset, since increased recurrence risk (usually distant metastasis) peaks between the 1st and 3rd year after therapy. The majority of deaths occur in the first five years after treatment, and the heterogeneity according to such outcome occurrence between triple-negative BC and non-triple-negative tumors decreases when the follow-up period is 10 years [4,5,7,15,35-38]. Patients with triple-negative and/or basal-like BC have a significantly lower survival after the first metastasis occurrence, even when compared to non-basal-like and/or non-triple-negative patients [4,7,15,35,36,38]. This more aggressive clinical behavior and poor prognosis are probably due to the biological tumor aggressiveness and resistance to currently available therapy. Triple-negative BCs preferentially exhibit hematogenic dissemination, especially to liver, lungs and central nervous system, and develop metastasis to axillary lymph nodes and bones less frequently than non-triple-negative BC [15]. In this study, a similar scenario was observed. Analyzing survival data at 12, 36, 60, 96 and 120 months, a statistically significant difference between 5-year overall survival curves was observed between triple-negative IDC and non-triple-negative IDC patients, respectively, 69% and 82.2%, log rank=6.3, *P*=.01. The magnitude of difference in five-year survival rates between patients with triple-negative IDC and non-triple-negative tumors was indeed higher and statistically significant comparatively to those observed in 10-year survival rates in both groups, (*P*=.058). For triple-negative IDC patients, 59% of deaths occurred within the first three years of follow-up, and 82% in the first five years of disease (38% and 65%, respectively, in non-triple-negative IDC patients).

The five-year overall survival found among triple-negative patients in the current investigation (69%) was quite similar (70%) to that reported in a study with 1,711 triple-negative BC patients, 93.6% of whom had tumors measuring 5.0 cm or less, and 67% had negative lymph nodes [9]. Data comparison between both studies revealed that a poor prognosis occurred in about 30% of patients from these cohorts, regardless of tumor size at diagnosis and the presence of axillary lymph node involvement. For this purpose, it is worth mentioning that in this Brazilian cohort, all patients had negative axillary lymph nodes and 48% had tumors measuring more than 5.0 cm. Nevertheless, in another investigation [8], based on 282 patients with triple-negative BC and 1,444 patients with non-triple-negative BC, both groups had a similar percentage of metastatic spread to axillary lymph nodes (37%), with most tumors measuring more than 1.5 cm. The authors reported survival estimates that were much higher than those found in the present study: 5-year and 10-year overall survival of about 80% and 75%, respectively, for triple-negative BC (versus 69% and 61.6%, respectively, in the present study), and about 90% and 85%, respectively, for non-triple-negative tumors (versus 82.2% and 70.1%, respectively, in the present study).

Most studies in the literature have revealed that triple-negative and/or basal-like BCs occur more frequently in women under 50 years, usually with large-sized tumors, IDC-NST histological type and high histological grade (grade 3) [4, 9, 14, 35, 36, 38]. The results obtained in the present study are very similar to those found in the literature: 45% of patients with triple-negative IDC were younger than 50 years of age (versus 27% of patients with non-triple-negative IDC; *P*=.001); 48% of patients with triple-negative IDC had tumors greater than 5.0 cm (versus 23% of patients with non-triple-negative IDC; *P*<0.001); 62% of triple-negative IDC were high histological grade tumors (grade 3), versus 21% of non-triple-

negative, *P*<0.001). Furthermore, while 22% triple-negative IDC patients were treated with preoperative chemotherapy, only 6% triple-negative IDC patients received the same type of treatment (*P*<0.001). The reason for this difference is probably the large-sized tumors in the former. Lerma et al. (2007) studying 64 triple-negative patients also reported the same mean age of the current investigation, 53 years [39].

Although just showing a non-statistically significance (*P*=.07) on survival (Kaplan-Meier analysis), age was tested in the Cox model for 95 patients with triple-negative IDC, considering its biological relevance to these tumors. According to the first ascertained Cox model for triple-negative IDC patients, an estimated two-fold higher risk of death among patients aged 50 years or older was verified (HR=2.02, 95%CI=1.00-4.09). In the second Cox model with the same group of triple-negative IDC patients, a 2.74-fold increased risk of death was estimated for patients aged 50 years or older (HR=2.74, 95%CI=1.23-5.78).

In a study by Albergaria et al.[15], the Nottingham prognostic index (NPI) and its components separately, tumor size and histological grade, in addition to axillary lymph node status [NPI = 0.2 x tumor size (cm) + histological grade (1-3) + nodal status (1-3)], were independent prognostic factors for the 10-year survival of patients with triple-negative BC. Using the Kaplan-Meier analysis, these authors found a 10-year overall survival of roughly 40% in triple-negative BC patients, either in those whose tumors had high NPI scores (> 5.4) or in those whose tumors measured more than 5.0 cm and had more than three positive lymph nodes. The 10-year overall survival was around 65% for patients with NPI scores ranging from 3.4 to 5.4. In the Cox regression model, the authors found that patients with triple-negative BC and tumors larger than 5.0 cm faced a 3.2-fold risk of dying from BC than patients with tumors smaller than 5.0 cm. In the present study, 48% of triple-negative IDC were larger than 5.0 cm, while 23% of non-triple-negative were of the same size (*P*<.001). The Kaplan-Meier analysis showed that the 10-year overall survival of patients with triple-negative IDC was higher (77%) in patients whose tumors measured 5.0 cm or less, in comparison to overall survival (45%) of patients whose tumors were larger than 5.0 cm (*P*=.004). However, in the Cox multivariate model, this variable lost statistical power. Regarding histological grade, the 10-year overall survival was 78% in patients with low-grade triple-negative IDC (histological grades 1 + 2) and 52% in high-grade triple-negative IDC (histological grade 3) (*P*=.02). For patients with non-triple-negative IDC, survival was 73% for low-grade tumors and 59% for high-grade tumors (*P*=.02). In one of the Cox model, the high histological (grade 3) was an independent prognostic factor only for the survival of patients with triple-negative IDC (*P*=.02). According to some authors, although the histological grade of the majority of triple-negative and/or basal-like BCs is high, the histological grade of up to 10% of these tumors may be low (grade 1) [4,5,35]. In the case study presented here, grade 1 corresponded to only 4(4%) cases.

In the literature research, the presence of vascular invasion in triple-negative BC ranged from 24 to 37% [4, 8, 9]. While some studies [8, 36] state that an association between triple-negative BC and vascular invasion is not frequently observed, other authors have reported a statistically significant association between both the five-year overall survival rate and disease-free survival [9]. In the present IDC series, only an association between vascular invasion and overall survival was found in a group of patients with non-triple-negative IDC, which remained an independent prognostic factor in the Cox regression model.

According to some authors, triple-negative and/or basal-like BCs exhibit a high Ki-67 expression and high p53 expression on immunohistochemical study (and/or high rate of TP53 gene mutation) [9,15,36,37]. In this cohort study, 63% of triple-negative IDC were Ki-

67 positive (high proliferation index), versus 53% of non-triple-negative IDC (*P*=.07). The 10-year overall survival was higher (78%) in patients with triple-negative IDC and Ki-67 negative tumors than in those with Ki-67 positive tumors (53%). In the Cox regression model, Ki-67 was an independent prognostic factor for 10-year overall survival (*P*=.01). A larger proportion of p53 positive tumors (37%) was found in triple-negative IDC compared to non-triple-negative IDC (18%), *P*<.001. On survival analysis, the p53 variable was statistically significant only for non-triple-negative IDC: survival rate was higher (73%) in patients with p53 negative tumors than in those with p53 positive tumors (57%), but statistical significance, however, was lost in the Cox regression model.

Dookeranet al., 2012 [40] concluded in their paper that “among women with breast cancer of different race/ethnicity, an adverse prognostic effect as a result of p53 positivity was only observed in African American women”. In our investigation, only 35% of patients were non-white (data not shown), but a comment on this topic is needed. Differently than in USA, ethnic mixing has been intense in Brazil since colonial times. Therefore, the usual ethnic stratification of whites and non-whites among Brazilians can be considered a quite inaccurate procedure, confirmed even by genetic studies such as Parra et al., 2003 [41].

The current study has some limitations. The data analyzed were obtained from two previous studies [42,43], in which an extensive histopathological review was undertaken for tumor reclassification and grading (only IDC were included), in addition to the study of other histopathological variables (skin involvement and vascular invasion). Review of gross anatomy or histological investigation and quantification of the presence of necrosis, fibrosis and/or peritumoral inflammatory infiltration were of no concern at the time. These were important aspects to characterize triple-negative BC [4,10,25,36,37], which unfortunately could not be evaluated in the present study. Immunohistochemical slides were not now reviewed, due to discoloration, and definitions of tumor marker positivity were not modified. During the performance of immunohistochemical studies (1997 - 2001), a cutoff point of 10% was used to define hormone receptor positivity. However, the American Society of Clinical Oncology and the College of American Pathologists currently recommend that the cutoff point be 1% [44], as other authors who defined the same cutoff point for patient selection in future clinical trials on triple-negative BC [38]. It is likely that the cutoff points used for both ER/PR and HER-2 in this study may have contributed to the high percentage of triple-negative IDC found: 27% against 10 to 20% in most studies in the literature [9, 13, 34, 36]. Additionally, since presently 14% is the cut-off used for Ki-67 proliferation index in breast cancer, the use of the 20% cut-off of Ki-67 [29,30] for classification as “low proliferation” could represent a potential limitation: it can be supposed that those cases settled between 14% and 20%, which had been previously considered as “low proliferation” (Ki-67 negative), will be now considered as “high proliferation” (Ki-67 positive).

Another factor that may have also contributed to the high percentage of triple-negative tumors in the present study was the exclusive use of IDC without metastasis to axillary lymph nodes, since most triple-negative BCs are IDC [4,36] and these tumors tend to produce less metastasis by the lymphatic route than non-triple-negative tumors [15]. The development of relapse and/or the presence of distant metastasis or metastasis sites, characteristics that are also important in triple-negative BCs were also not investigated in patient medical charts. The chemotherapy regimen used at the time (preoperative and/or post-operative) was also not the aim of this study. Chemotherapy regimen was probably much different from the currently used regimen.

In contrast, this study has some positive aspects. To our knowledge, this was so far the first Brazilian investigation conducted to determine the 10-year survival in cases of triple-negative IDC in the country. Furthermore, all cases were diagnosed, treated and followed in a national referral center for oncology treatment, ensuring homogeneity among therapeutic procedures in the post-diagnostic period, prior to the existence of BC classification based on immunohistochemical phenotype. Finally, the results obtained from the present study were similar to those found in many studies described in the literature, so this reproducibility tends to suggest that estimates from this study have not been biased.

Results obtained from this study indicate that rapid identification of triple-negative tumors is important. The adoption of prompt measures of therapeutic interventions that can alter the natural history of triple-negative tumors and their effects on mortality rates of affected women should be prioritized.

4. CONCLUSION

Breast cancer with a triple-negative immunophenotype identifies a group of BC with more aggressive behavior than the remaining subtypes of tumors. The development of appropriate targeted therapy and a better understanding of the prognostic factors are mandatory.

Basal-like BC used to be recognized by genetic analysis, but currently, immunohistochemistry has been added to identify these tumors. However, an international consensus on the best markers to be adopted still lacks.

The authors have shown that patients with triple-negative IDC from this study had a worst prognosis and consequently a worst overall survival, when compared to those with non-triple-negative IDC (particularly in the first five years of follow-up). The independent factors with worse prognosis associated with 10-year overall survival in this Brazilian cohort with triple-negative IDC were age 50 years or older, treatment with preoperative chemotherapy, skin involvement, high histological grade (grade 3), and Ki-67 positive tumors. For non-triple-negative IDC, the worst prognostic factors were skin involvement and vascular invasion.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (Research Ethics Committee of INCA under number 109/2011) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

Authors would like to thank to Sophie Derchain (UNICAMP, Brazil) and Fernando Schmitt (IPATIMUP, Portugal) for having read and criticized the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=205&id=12&aid=1077