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EDITORIAL
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Breast cancer care during the coronavirus pandemic

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The coronavirus disease 2019 (COVID-19) is caused by the virus SARS-CoV-2, a new coronavirus detected in December 2019 in Wuhan, China.[1] Due to its highly contagious nature, the disease quickly spread over the world, and, on March 11, 2020, the World Health Organization declared the infection outbreak as the first pandemic caused by a coronavirus.[2] On April 17, 2020, COVID-19 had reached 210 countries, infected over 2.2 million people, and caused more than 150 thousand deaths.[3] Most infected individuals develop mild to moderate respiratory symptoms; however, older adults or those with health conditions, such as diabetes mellitus, cardiovascular disease, hypertension, chronic respiratory disease, chronic kidney disease, and immunodepression, may present severe forms of COVID-19 and require intensive medical care, with hospitalization and clinical and ventilatory support. It is worth mentioning that cancer patients are more susceptible to infections, either by the immunosuppressed state inherent to the disease or the necessary antiblastic treatment, such as chemotherapy, targeted therapy, and immunotherapy.[1]

In order to preserve and provide essential resources to fight the pandemic, public and private hospital services are forced to reduce the supply for routine care. Thus, patients and physicians must adapt to this new reality, seek protection against contamination in the work environment, and understand that the number of beds available for elective hospitalizations and emergency treatments is low. In addition, the cancer patient faces a higher risk of contamination by the new coronavirus in a saturated hospital environment. Yu et al. reviewed data from 1,525 cancer patients treated at a tertiary hospital in Wuhan, comparing the incidence of COVID-19 in these individuals with that of the general local population, and noted that the risk of infection by SARS-CoV-2 was significantly greater among the first group (odds ratio – OR=2.31; 95% confidence interval – 95%CI 1.89–3.02).[4]

In recent weeks, much has been discussed about adjustments to the care of cancer patients not infected by the new coronavirus during the pandemic to minimize the risk of contamination, without compromising the outcome of the disease. Some associations summarized recommendations that should be periodically adapted, given the rapid dissemination of COVID-19 and the local availability of resources.[4,5]

RECOMMENDATIONS FOR THE CARE OF BREAST CANCER PATIENTS DURING THE COVID-19 PANDEMIC

- Adopt the use of telemedicine (Office Letter from the Federal Council of Medicine no. 1,756/2020, March 19, 2020) on an exceptional basis during the fight against the COVID-19 for the remote instruction of patients in isolation, medical supervision of health parameters and/or disease, and exchange of information and opinions among physicians;[6]
- Schedule appointments with greater interval to reduce the contact between individuals in the waiting room;
- Decrease the number of companions in appointments;
- Keep a safe distance between the patient and health professionals;
- Do not make greeting gestures;
- Wash and sanitize the hands before and after the physical examination;
- Always use disposable gloves during the physical examination;
- Inform the patient about the signs and symptoms of COVID-19;
- Counsel the patient on social distancing and day-to-day hygiene;
- Offer the diagnostic test for the symptomatic patient;
- Postpone elective surgeries when possible. The decision should be individualized, based on common sense, multidisciplinary, and shared with the patient. The surgeries indicated must respect the hospital resources available, depending on the phase of the pandemic. In the initial phase (phase I) of the COVID-19 pandemic in a region, the hospital resources are still reasonable. Thus, patients who would have their survival impaired if not operated within the next three months should undergo surgery. Patients who have non-urgent surgeries postponed should be informed that the decisions was made by consensus and based on local resources, due to the prevalence of COVID-19, as well as the characteristics

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of the tumor and the expected results related to the delay. All information and instructions must be included in the medical records. In the next phase (phase II), hospital resources are scarce, with a limited number of respirators and intensive care unit beds. Surgeries are restricted to patients who would not survive a few days if not operated. Among these conditions, abscess drainage, hematomas, and review of flap ischemia (reconstructions with autologous flaps must not be performed) stand out. In phase III, no respirators or beds are available for admission. Virtually all hospital resources are consumed. At this stage, the surgeries are restricted to patients who would not survive a few hours if not operated;

- Postpone, discontinue, or modify the radiotherapy, when possible, depending on the risk of contamination and the clinical indication;
- Individualize the systemic therapy, grounding the measure in the likelihood of recurrence. Some patients can receive home infusions or change intravenous for oral therapy to reduce the number of visits to hospital units.

In short, the pandemic caused by the new coronavirus SARS-CoV-2 has an uncertain trajectory and represents a great challenge both economically and emotionally.[7] It is the moment to learn and prepare for the huge impact that this outbreak might have on the appropriate support to cancer patients.

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ORIGINAL ARTICLE
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Correlation between the proportion of healthy mammary tissue versus tumor size in breast-conserving surgeries

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ABSTRACT

Objective: To evaluate the proportion of excised healthy tissue in breast-conserving surgeries and to identify possible tendency toward excision in healthy tissue beyond the ideal for oncological safety. **Methods:** Data from patients who underwent breast-conserving surgery at the Hospital Geral de Caxias do Sul from January 2010 to December 2016 were analyzed. For statistical purposes, means, standard deviations, Student's t-test, and linear regression were used for numerical variables. Risk estimate by odds ratio (OR) was performed through logistic regression with 95% CI. A significance level (alpha) of 5% was adopted. **Results:** A total of 124 cases were analyzed. The mean tumor size observed by ultrasonography was 1.7 ± 0.95 cm. The tumor size by pathology was 1.9 ± 1.12 cm. The mean size of the resected surgical specimens was 7.8 ± 3.4cm. When comparing the tumor size in the anatomopathological examination and the size in ultrasonography, the mean differences accounted for 0.6 cm (95%CI -0.10–0.44; p = 0.2). Conversely, the difference in the size of the total surgical specimen versus tumor size in the anatomopathological examination was 5.8 cm (95%CI 5.2–6.5; p < 0.001). There was no statistical difference regarding the tumor location nor size of the surgical specimen. **Conclusion:** It was observed that there is a tendency toward excising a large amount of healthy tissue in breast-conserving surgeries far beyond what is recommended in order to consider the oncological safety of excised margins.

KEYWORDS: mastectomy, segmental; margins of excision; breast neoplasms; treatment outcome; esthetics.

INTRODUCTION

Breast cancer is the tumor that most affects women worldwide. In Brazil, breast cancer mortality rates remain high, probably because the disease is still diagnosed in advanced stages. Population screening programs enabled more diagnoses of early-stage injuries, reducing death cases and promoting less aggressive surgeries[1]. The José Alencar Gomes da Silva Brazilian National Cancer Institute (*Instituto Nacional de Câncer* – INCA) estimated 59,700 new cases of breast cancer in Brazil in 2018[2]. In Caxias do Sul, in the state of Rio Grande do Sul, 46 cases of death from breast cancer were identified in 2016[3].

Surgical treatment of breast cancer has undergone significant changes in recent decades, and breast-conserving surgery is the standard treatment for the early stages of the disease nowadays[4]. The radical mastectomy technique and its corresponding lymphatic drainage have been abandoned. The old Halstedian paradigm had been overcome, and conservative treatments, both for the excision of breast tissue and for the surgical approach of the armpit, have been increasingly employed[5,6].

The theory proposed by Bernard Fisher, which defines breast cancer as a systemic disease, was the basis for the development of breast-conserving surgery, providing a new and much-less aggressive perspective to surgical therapy[7-9].

Veronesi, author of the renowned *Milan I* study, conducted between 1973 and 1980, analyzed 701 cases of early-stage breast cancer and randomized a group to undergo breast-conserving surgery with radiotherapy and another group with radical mastectomy[10]. After 20 years of follow-up, the author observed that both

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groups obtained the same long-term survival rates. This study revolutionized breast cancer treatment, making breast-conserving surgery a treatment chosen for early-stage cases[11].

Nowadays, most patients in stages I and II of the disease are candidates for breast-conserving treatment, which consists of undergoing surgery with partial excision of the mammary gland (sectionectomy or quadrantectomy) followed by radiotherapy[1]. For this surgical decision, tumor size is not an exclusive limiting factor of conservative surgery. The tumor-to-breast volume ratio is the most important anatomical factor. Thus, breast-conserving surgery must always be the first option, provided that there are no contraindications to the procedure and that the tumor-to-breast volume ratio allows a surgical excision with satisfactory cosmetic outcome, according to oncological surgery concepts[12].

Therefore, it is established that the aim of breast-conserving surgery is to completely remove the tumor with free margins, obtaining a good cosmetic result, but without compromising local recurrence rates[1].

Prospective, randomized clinical trials have shown that there is no significant difference in distant disease-free survival or overall survival between patients treated with mastectomy and those treated with breast-conserving surgery and radiotherapy. This reinforces the indication of breast-conserving surgery as the best cosmetic alternative for most patients, since it provides the same cure rates without the aggressiveness and mutilation caused by mastectomy[9,11]. However, 4 to 20% of patients with early-stage breast cancer have local recurrence[13].

The lack of adjuvant radiotherapy and positive surgical margins was associated with an increase in this recurrence[13,14]. In addition, it is known that local recurrence increases the risk of distant recurrence[15,16]. Compromised surgical margin is the most common indication of reexcision after breast-conserving surgery, and this approach can lead to worse cosmetic results, increased risk of infection, higher costs, and delay in early adjuvant treatment[1].

There is an intense debate about surgical margins, although the 2010 International Consensus defines positive margin as ink on microscopic tumors in cases of invasive carcinomas and a 2-mm margin for carcinoma *in situ*[16,17].

Factors, such as tumor biology and the availability of effective systemic therapy, are as important as the margin of microscopic residual disease in determining local control. The standard definition of negative margin as no ink on the tumor has the clear potential to decrease the indication for surgical reexcision, in addition to avoiding large resections that often require additional remodeling surgery of the affected breast and even of the contralateral breast for symmetry purposes[17,18].

Over the years, the idea that the lower the volume of excised healthy tissue, the greater the probability of incomplete removal of the neoplasm has been promoted. Likewise, there would be a greater probability of local recurrence due to the growth of the remaining neoplasm. However, the higher the volume of excised breast tissue, the lower the chances of obtaining more satisfactory cosmetic results[12].

Waljee et al. conducted a study in which they evaluated the aesthetic effect perceived by patients after breast-conserving surgery, and demonstrated that large asymmetries were correlated with depressive symptoms and worsening in the psychosocial functioning and quality of life of these women[19].

Thus, considering the importance of the theme, the present study aimed to identify possible tendencies toward excision in healthy tissue beyond the ideal for oncological safety. The results observed here can be used to produce recommendations regarding the volume of tissue to be excised, aiming at cosmesis and aesthetics without impairing the oncological conduct for breast surgeries.

METHODS

This is a cross-sectional and retrospective study conducted at the Mastology Center of Hospital Geral de Caxias do Sul, in the state of Rio Grande do Sul, Brazil. The medical records of all patients who underwent breast-conserving surgery at the institution, from January 2010 to December 2016, were analyzed.

Eligibility criteria were considered for patients who underwent breast-conserving surgery (sectionectomy or quadrantectomy) and who had a diagnosis of cancer at the time of surgery or cases already confirmed prior to the procedure (prior biopsy).

Data on incomplete or dubious medical records, multicentric/multifocal tumors, and patients submitted to surgical reintervention to enlarge margins were deemed reasons for exclusion from the study.

Data were compiled and evaluated after surveying medical records by research members. The following categories were analyzed: age; menopausal status; tumor size on ultrasonography; tumor size on anatomopathological examination; size of the excised surgical specimen; excised healthy tissue; free or not surgical margin; number of compromised axillary lymph nodes; chemotherapy; tumor location; and histological and molecular characteristics.

Due to the heterogeneity of information in the medical records, the tumor size for the anteroposterior diameter in ultrasound and anatomopathological examination and the size of the excised tissue were considered for comparison purposes.

For patients undergoing neoadjuvant chemotherapy, the residual tumor size after chemotherapy treatment was taken into account.

In the analysis of surgical margin, the disease-free surgical margin was established as no ink on the tumor in cases of invasive tumors and margins greater than 2 mm in cases of tumors *in situ*.

Data analysis

For statistical purposes, means, standard deviations, Student's t-test, and linear regression for numerical variables were used.

A risk estimate was carried out by odds ratio (OR) through logistic regression with a 95% confidence interval (95%CI). Significance level (alpha) of 5% was adopted.

The database was submitted to a double-entry process with inconsistency processing. Moreover, multivariate backward linear logistic regression was used, associating the new variable with those previously reported. P-value < 0.05 was deemed statistically significant. Analyses were performed using R 3.1.1 for Windows (R-Cran project), with the MASS package for Windows.

The study was submitted to and approved by the Research Ethics Committee of Universidade de Caxias do Sul (UCS).

RESULTS

Of the total of 194 breast-conserving surgeries performed from January 2010 to December 2016, and according to the inclusion and exclusion criteria, 124 patients remained in the study. The other cases were excluded due to reexcisions, subsequent surgeries related to margin enlargement and multicentric or multifocal tumors, and those related to incomplete hospital data.

Table 1 summarizes the characteristics and results obtained in the present study. In the study group, 56.9 ± 11.7 was the mean age in years. Considering menopausal status, 33 patients (26.6%) accounted for premenopausal status, and 91 of them (73.4%) accounted for postmenopausal status at the time of diagnosis.

Regarding the axillary status, 92 patients (74.2%) had negative axillary lymph nodes, 24 (19.3%) had 1-3 lymph nodes compromised by neoplasia, and 8 (6.5%) had more than four affected lymph nodes.

It was identified that 59 patients did not undergo chemotherapy. Of the 65 patients who did it, 48 were adjuvant and 17 were neoadjuvant.

Regarding the pathological characteristics of the tumors, 70 cases (56.5%) were of no special type (invasive ductal); 18 (14.5%) had invasive ductal carcinoma and concomitant ***in situ;*** 14 cases (11.3%) were of special subtypes (e.g., tubular, medullary, mucinous, papillary, etc.); 13 (10.5%), ductal carcinoma ***in situ;*** and 5 cases (4%) of invasive lobular carcinoma. Four (3.2%) tumors exhibited histological types other than those aforementioned.

As for molecular classification by immunohistochemistry, 56 tumors (45%) were of the type Luminal A; 48 (39%), Luminal B; 11 (8.8%), human epidermal growth factor receptor 2 (HER2); and 7 (5.6%), triple-negative breast cancer. In two cases, immunohistochemistry was not performed because they were nonepithelial tumors (1.6%).

In Table 2 and Graph 1, one may observe the distribution of tumors regarding the location in the breast and the mean of excised tissue. There was no statistical difference regarding tumor location and neither concerning the size of excised tissue in the surgical specimen.

The mean tumor size observed by ultrasonography was 1.7 ± 0.95 cm. The tumor size in the anatomopathological examination was 1.9 ± 1.12 cm. Conversely, the mean size of the excised surgical specimens was 7.8 ± 3.4cm.

Table 3 and Graph 2 show the amount of excised tissue according to tumor size (in the anatomopathological examination). When comparing groups 1, 2, and 3 with group 4, there was an increase in the resected tissue in group 4 with statistical difference (p < 0.01).

When comparing the tumor size in the anatomopathological examination and the size in ultrasonography, the mean differences accounted for 0.6 cm (95%CI -0.10–0.44; p = 0.2).

Table 1. Clinical and demographic characteristics of patients included in the study (n = 124).

Characteristic	Value	N	(%)
Menopausal status	Premenopausal	33	26.6
	Postmenopausal	91	73.4
Axillary status	Negative	92	74.2
	1–3 positive	24	19.3
	> 4 positive	8	6.5
Histological type	NST	70 cases	56.5
	NST + DICS	18 cases	14.5
	Special subtypes	14	11.3
	DCIS	DCIS	10.5
	10.5	5	4
	Other types	4	3.2
Immunohistochemistry	Luminal A	56	45
	Luminal B	48	39
	HER2	11	8.8
	Triple-negative	7	5.6
	No tests	2	1.6
Characteristic	**Value (mean with SD)**		
Age	56.9 ± 11.7 years		
Tumor size in US	1.7 ± 0.95 cm		
Tumor size in AP	1.9 ± 1.12 cm		
Size of the surgical specimen	7.8 ± 3.4 cm		

US: ultrasound; AP: anatomopathological examination; NST: invasive ductal carcinoma (of no special type); DCIS: ductal carcinoma *in situ*; ILC: invasive lobular carcinoma; HER2: human epidermal growth factor receptor 2; SD: standard deviation.

Table 2. Location of tumors and mean excised tissue.

Quadrants	N (%)	Excised size	95%CI
UOQ + JUQ	70 (56.5)	8.1 cm	7.5–9
LOQ + JOQ	21 (16.9)	6.7 cm	5.5–8.2
UIQ + JIQ	13 (10.5)	6.3 cm	4.5–8.2
LIQ + JLQ	17 (13.7)	8.4 cm	7–10.2
RA	3 (2.4)	5.6 cm	1.8–9.5

UOQ + JUQ: upper outer quadrant + junction of the upper quadrants; LOQ + JOQ: lower outer quadrant + junction of the outer quadrants; UIQ + JIQ: upper inner quadrant + junction of the inner quadrants; LIQ + JLQ: lower inner quadrant + junction of the lower quadrants; RA: retroareolar region; 95%CI: 95% confidence interval.

On the other hand, the ratio between the size of the total surgical specimen and the tumor size in the anatomopathological examination accounted for 5.8 cm (95%CI 5.2–6.5; p < 0.001).

In all cases, free surgical margins were obtained, as established by the literature.

DISCUSSION

Breast cancer is relatively rare before the age of 35, and its incidence progressively increases above this age, especially after 50 years of age[2]. The age group of patients in our study ranged from 27 to 77 years (mean of 56.7 ± 11.7 years), and most (73.4%) were postmenopausal.

The development and evolution of the sentinel-lymph-node biopsy have positively affected the treatment of early-stage breast cancer. This procedure provides accurate diagnosis and prognostic information on patients with clinically negative lymph nodes and consists of a primary tool to guide surgical and adjuvant treatment. In many cases, sentinel-lymph-node biopsy has replaced axillary dissection, and patients were spared of lymphedema and additional morbidity attributed to this procedure, thus improving their quality of life[20].

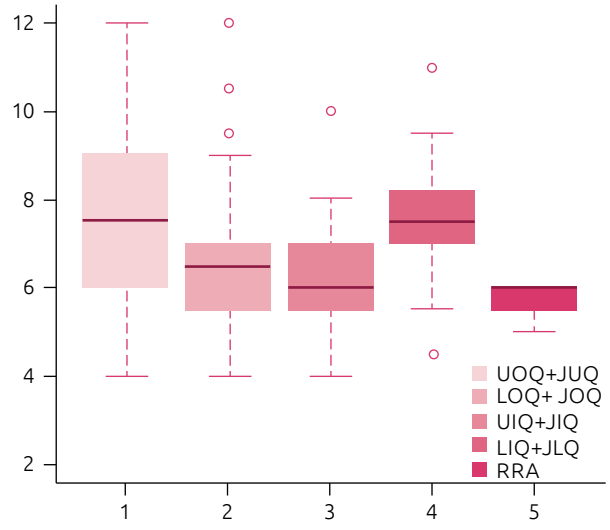
In the present research, 92 patients (74.2%) had negative axillary lymph nodes; 24 (19.3%) had 1-3 lymph nodes compromised by neoplasia; and only 8 (6.5%) had more than four affected lymph nodes. Since this study only analyzed breast-conserving surgeries and, therefore, patients with early-stage cancer, most patients did not present lymph node metastases.

Veronesi et al. analyzed patients with tumors < 2-cm who were submitted to sentinel-lymph-node investigation, and found that 65% of them presented negative lymph nodes at the time of the surgery[21].

A Korean study, whose authors analyzed 945 patients with breast cancer in stages I and II, showed that the molecular subtype is a prognostic factor as important as the compromise of lymph nodes[22]. In this same study, the most frequent subtypes, in order, were Luminal A (41%), Luminal B (29.1%), triple-negative (21.6%), and HER2 (8.3%). In our study, Luminal A and Luminal B were also the majority, but there were more cases of HER2 than triple-negative.

Invasive ductal carcinoma of no special type is the most common histological type, corresponding to 40–75% of breast carcinomas, depending on the series evaluated, and invasive lobular carcinoma accounts for 5–15% of invasive carcinomas[23]. The findings of this research showed that the invasive ductal carcinoma of no special type corresponded to 56.5% of cases, and the invasive lobular corresponded to 4%, corroborating data presented in other studies.

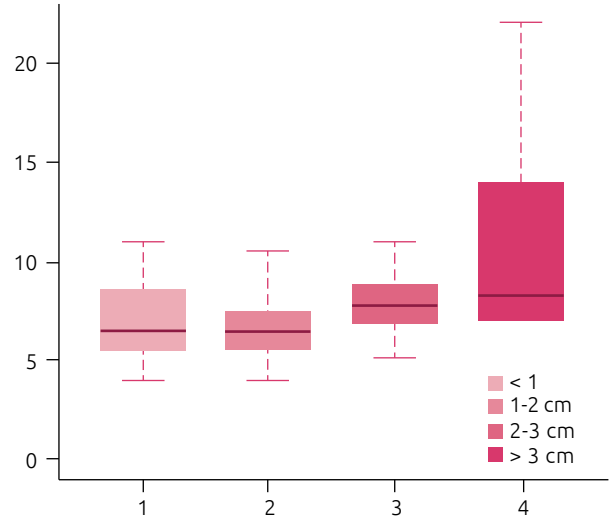
The authors identified 70 cases (56.6%) of tumors located in the upper outer quadrant or junction of the upper quadrants, which are quadrants where there is a higher volume of breast



Graphic 1. Size of the surgical specimen *versus* tumor location. UOQ + JUQ: upper outer quadrant + junction of the upper quadrants; LOQ + JOQ: lower outer quadrant + junction of the outer quadrants; UIQ + JIQ: upper inner quadrant + junction of the inner quadrants; LIQ + JLQ: lower inner quadrant + junction of the lower quadrants; RA: retroareolar region.

Table 3. Tumor size ***versus*** excised tissue size.

Group	Tumor size	Excised size (mean)
1	< 1 cm	7.2 cm ± 0.55
2	1 to 2 cm	6.94 cm ± 0.71
3	> 2–3 cm	7.83 cm ± 0.81
4	> 3 cm	11.42 cm ± 1.0



Graphic 2. Size of surgical specimen *versus* tumor size.

tissue and, therefore, are more likely to develop the neoplasm. There was no statistical difference regarding tumor location and neither concerning the size of excised tissue in the surgical specimen.

The mean tumor size was 1.9 ± 1.12 cm, a result similar to that found in other studies whose authors analyzed patients with early-stage breast cancer[24,25].

With the increased use of neoadjuvant chemotherapy and breast-conserving surgery, the accuracy of preoperative tumor size assessment has become important for assisting in the therapeutic decision. Tests such as ultrasound, mammography, and magnetic resonance imaging, can be used for this purpose. Studies have shown that ultrasound is better than mammography for estimating tumor size[26]. When comparing ultrasound and mammography with magnetic resonance imaging, the latter is the most accurate method[27]. When comparing tumor size in anatomopathological examinations and its size in ultrasonography, the mean difference of 0.6 cm (95%CI -0.10–0.44; p = 0.2) was identified.

Authors of other studies have also observed differences, such as Shoma et al., who compared the evaluation of tumor size by physical examination, mammography, and ultrasound and found a mean difference of 3.2 ± 0.4 mm[28] in size between ultrasound and anatomopathological examination.

It is clearly perceived that larger tumors dictate techniques that ultimately excise a greater amount of healthy tissue. When comparing groups 1, 2, and 3 with group 4, there was an increase in the size of excised tissue in group 4, with statistical difference (p< 0.01). This shows the clear tendency of surgeons for being more aggressive, even in conserving surgeries, when operating tumors whose mean diameter is greater than 3 cm.

The tumor-to-breast volume ratio does not become an absolute contraindication to breast-conserving surgery, provided that it is possible to excise the tumor area, maintaining oncological safety, and causing no large asymmetries[12]. Taking this into consideration, patients with large tumors and small breasts are not likely to be submitted to breast-conserving surgery. Conversely, patients with more voluminous breasts consequently allow for greater tissue resection without major aesthetic impairments, which may justify our findings.

The difference in the size of the total surgical specimen and the tumor size in the anatomopathological examination accounted for 5.8 cm (95%CI 5.2–6.5; p < 0.001). When performing simple linear regression, it was observed that every 1 cm of tumor in the anatomopathological examination corresponds to 6.7 cm of surgical tissue.

This finding demonstrates that excessive and unnecessary healthy tissue is being excised in order to obtain a disease-free surgical margin. One possible reason for explaining excessive resection is the attempt to avoid subjecting the patient to a new surgical procedure to enlarge the margins, thus delaying the onset of adjuvant therapy.

The need to obtain disease-free surgical margins is due to the fact that this is the most important factor in reducing the risk of local recurrence[29]. It is known that ¼ of patients undergoing breast-conserving surgery will require a new surgical procedure for margin enlargement[30]. The use of frozen section histology assists in identifying margins compromised during the intraoperative period, avoiding excessive tissue excision or other surgery, providing more comfort and agility to the surgeons, since they will have information on enlargement of margins in appropriate time for doing it so, which also enhances the chances for surgeries seeking to conserve more healthy tissues.

Nevertheless, this evaluation technique is not a standard procedure in all services, and some authors suggest that the tool may alter the pathological staging and is contraindicated in some cases, such as in small tumors. In addition, the definition of complete excision of the tumor with safety margins is only provided after a histological study of the surgical specimen embedded in paraffin[12].

Another reason that could explain excessive excision of healthy tissue is the fact that patients with large breasts have greater possibility of wide resection with minor aesthetic defects; however, the purpose of this study was not to evaluate the preoperative breast volume.

CONCLUSION

It was observed there is a tendency toward excising a large amount of healthy tissue in breast-conserving surgeries, far beyond what is recommended in order to consider the oncological safety of excised margins. The excessive excision of healthy tissue found in this study can bring severe deformities to the breast. An unfavorable aesthetic result may generate emotional impairment and compromise the patients' quality of life, thus opposing the main objective of breast-conserving surgery, which is to maintain cosmesis without harming the oncological conduct.

AUTHORS' CONTRIBUTIONS

G.P.: Conceptualization, Data curations, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.
F.V.: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing - review & editing.
V. B.: Data curation, Investigation, Visualization.
J. P.: Data curation, Investigation, Visualization.

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Main prognostic and predictive immunohistochemical factors in breast cancer: a retrospective cohort study

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ABSTRACT

Introduction: Breast cancer is a constant focus of studies on prevention and treatment. Immunohistochemistry is a useful tool for defining the conducts toward the treatment of this disease. **Objective:** To evaluate patients' survival according to prognostic and predictive immunohistochemical factors. **Method:** This is a retrospective cohort study. Medical reports of 787 patients were analyzed, which contained parts of surgical specimens of the mastectomy or quadrantectomy procedures. A total of 404 patients were eligible for the study. **Results:** The mean age at diagnosis of the disease was 55.4 years. The main diagnosis was infiltrating ductal carcinoma (80.7%). Of the total, 45% of the patients had tumors of up to 2 cm in diameter, and 32.9% had lymph node involvement. Among the patients, and according to luminal molecular classification, 48.3% were classified as luminal A, 27% were luminal B, 12.1% were recipient of human epidermal growth factor type 2 (HER2), and 12.6% were triple-negative. Furthermore, of 23.3% patients with tumor recurrence, 12.6% of them died. The 1% increase in Ki-67 values increases the risk of death and recurrence by 2% and 1%, respectively. The presence of lymph node metastasis increases, on average, 4.78 times and 2.63 times the risk of death and recurrence, respectively. **Conclusion:** The triple negative molecular classification had the lowest overall survival and the greatest risk of recurrence. The luminal A classification presented the best prognosis. Tumor size, lymph node metastasis, skin invasion, and presence of Ki-67 were shown to be the prognostic and predictive factors that most influenced the patients' survival.

KEYWORDS: breast cancer; immunohistochemistry; prognosis; survival; recurrence.

INTRODUCTION

Breast cancer is the most common malignant neoplasm found in Southern Brazil, with the exception of non-melanoma skin cancer. In 2018 alone, there were 56.33 cases per 100,000 women, which corresponds to more than 20% of all types of cancer[1].

Breast cancer is the leading cause of death among women worldwide, accounting for 522,000 deaths in 2012 alone, equivalent to 14.7% of all deaths in that year. The incidence of breast cancer has virtually increased worldwide, but in developed countries, this number has decreased in the last 10 years. Moreover, there has been a reduction in the death rate related to breast cancer due to adequate screening, early detection, and effective therapy[2].

Breast neoplasm does not indicate clinical uniformity and is characterized according to the morphology of the disease, thus existing different molecular forms and subtypes. Instead, it should be stated that breast cancer consists of a range of distinct neoplasms, which are all classified as breast cancer. These varied forms of the disease enable the evaluation and development of prognosis based on their evolution, making it possible to prescribe specific treatments according to the development and characteristics of each type. Acknowledging this is important due to the need for defining the prognosis and the appropriate approach, aiming at avoiding to unnecessarily submit patients to aggressive treatments such as chemotherapy[3].

Immunohistochemical examination and anatomopathological analysis are paramount to define the disease approach and the prognosis of the patient. Immunohistochemistry is a technique used to identify biological characteristics of tumors, including breast-related ones. Molecular technology with biomarkers allows identifying and classifying breast cancer into different subtypes that, consequently, exhibit different behaviors. Biomarkers are often used for determining the best therapy to be provided and

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for other decisions concerning treatment approaches, including the confirmation of metastases. This technology has proved to be an important diagnosis tool , since it is a simple, practical, and versatile instrument[4].

PROGNOSTIC FACTORS

Prognostic factors consist of aspects that may interfere with the clinical evolution of the disease at the time of diagnosis. The main parameters for determining the therapeutic planning of breast cancer are age, tumor size, lymph node involvement, and molecular subtype[5].

Age is among the three main prognostic factors that are prominent when it comes to survival in breast cancer. It carries a considerable weight to decisions to be made at two moments during the course of the disease: first, at diagnosis and, secondly, at the definition of the treatment to be provided, being older age directly related to the worst outcome of breast cancer.[6] Older women and those in menopause have fewer recurrences and deaths from breast cancer, usually because they feature less aggressive molecular classification, though they are affected by age-related issues, and the presence of aging-related comorbidities, which limit therapies or their responses, are common. Conversely, younger women develop larger tumors, high histologic grade, increased vascular invasion, and lymph node involvement, even when submitted to more aggressive treatments[7-9].

Tumor size has key importance in the survival of breast cancer patients. Survival is proportionally inferior to tumor size. That is, tumors with larger diameters are associated with lymph node involvement, higher mortality, and lower disease-free survival[8-12].

Breast tumors manifest responses to the provided therapies and disease evolution in a very varied way. This is because breast tumors have complex genome variation. These variations allow such tumors to present very different evolutions and biological behaviors, although they are all classified as breast cancer. Molecular classification allows identifying, with a high degree of accuracy, different types of the disease based on profiles. Thus, if a metastasis, whether distant or in a lymph node, is related to a certain tumor, it will present the same pattern of genes as if it were a sample of the main tumor[13].

PREDICTIVE FACTORS

Lymph node involvement is the predictive factor that mostly influences therapeutic approaches. Based on this involvement, the breast volume that will be exposed to radiation in radiotherapy treatment can determine, in addition to whether there shall be lymph node clearance of the axillary region, which can cause important side and aesthetic effects on the quality of life of patients under treatment[14]. This factor greatly influences the outcome of breast cancer, especially when there is involvement of axillary lymph nodes, since they have a strong impact on overall survival and disease-free survival in a 10-year period[8,9]. Lymph node involvement indicates that, in addition to breast cancer being aggressive, it is already in a dimension that will interfere with disease-free and overall survival rates, regardless of the provided therapy[15].

Hence, lymph node invasion is a predictive factor for metastatic dissemination of breast cancer, contributing to a worsened evolution of the disease[16].

The most commonly used biomarkers in determining the treatment for breast cancer are estrogen and progesterone hormone receptors[17].

The human epidermal growth factor receptor type 2 (HER2) performs specific functions of cell differentiation, regulation, and proliferation. Its overexpression occurs in 15% of breast tumors. Mostly, it features negative hormone receptors and is related to a more aggressive type of the disease and worse prognosis. Its advantage is the current existence of target molecular therapy for tumors manifesting this overexpressed factor[18,19].

The Ki-67 proliferation index indicates cell multiplication. It is present in all active phases of the cell cycle, with the exception of the G0 phase[20], being routinely evaluated in immunohistochemical tests for breast cancer as it is responsible for the differentiation between tumors of luminal types A and B. Ki-67 is directly associated with tumor aggressiveness and poor prognosis[21]. It represents high histologic grade and high speed of tumor growth, providing reliable, easy-to-analyze, and low-cost information, being paramount for determining the clinical conduct[22].

Breast tumor cells have many structural differences, even when they are very similar according to microscope images. Immunophenotyping allowed the creation of gene expression profiling, which can be used to identify tumor evolution based on its molecular phenotype[7].

The aim of this study was to compare the main pathological prognostic and predictive factors with the outcome of patients who underwent treatments for breast carcinoma. Disease-free survival time was related to prognostic factors of tumor size, age, and lymph node involvement; in addition, disease-free survival time according to predictive factors of molecular classification by immunophenotyping were evaluated.

METHODOLOGY

A survey on all female patients who had their surgical specimens of breast carcinoma analyzed in the Pathology Laboratory of *Hospital Santa Rita da Irmandade da Santa-Casa de Misericórdia de Porto Alegre* (ISCMPA), from 2008 to 2012, was performed. Then, each of the medical reports were read, leading to the selection of those in which the specimens derived from a surgical procedure of mastectomy or quadrantectomy. Each of the medical reports was cataloged and transformed into a number, aiming to ensure the

patients' anonymity. Date of diagnosis, age of the patient, size of the surgical specimen, tumor grade, immunohistochemical classification, surgical margins, lymph node involvement, presence of carcinoma *in situ*, date of recurrence (when is the case), and date of the last follow-up were used to import data into a spreadsheet in the Excel computer program® for the analysis.

In some cases, there were divergences between the immunohistochemical classification of the biopsy and the subsequent analysis of the surgical specimen. This is due to biopsies being performed on a small portion of the tumor. On the other hand, the surgical specimen is analyzed in the so-called "hot spot," where the highest concentration of tumor cells is found. Since it is deemed the most reliable analysis, a real classification was considered as that performed after the analysis of the specimen by the Pathology Laboratory. The deadline for updating each patient's outcome was December 31st, 2018.

Death was measured and validated in the study only when it occurred within the institution and it was recorded in the electronic medical reports of each patient.

Patients who had undergone any procedure other than mastectomy or quadrantectomy, those with a history of previous neoplasms, or whose pathological examinations proved the emergence of new primary lesions were excluded from the study.

We followed the ethical precepts of Resolution No. 466/2012 of the National Health Council (*Conselho Nacional de Saúde* – CNS), respecting the confidentiality of the participating subjects. Data were anonymously managed, without any nominal identification or other information that allowed identifying the participants.

The project was approved by the Research Ethics Committee of ISCMPA, under Opinion no. 2.324.152.

STATISTICAL ANALYSIS

Quantitative variables were described by mean and standard deviation or by median and interquartile range, and categorical variables, by absolute and relative frequencies (Table 1).

Overall survival and disease-free survival curves were estimated by the Kaplan-Meier method[22] (Figures 1 and 2). To evaluate factors associated with outcomes, the univariate and the multivariate Cox proportional hazards regression models[23] were applied (Table 2). All variables that presented p<0.20 in the univariate analysis were inserted in the multivariate model (Table 3); in the final model, only variables presenting p<0.10 remained.

The adopted significance level was 5%, and analyses were performed in the Statistical Package for the Social Sciences (SPSS) program, version 21.0.

RESULTS

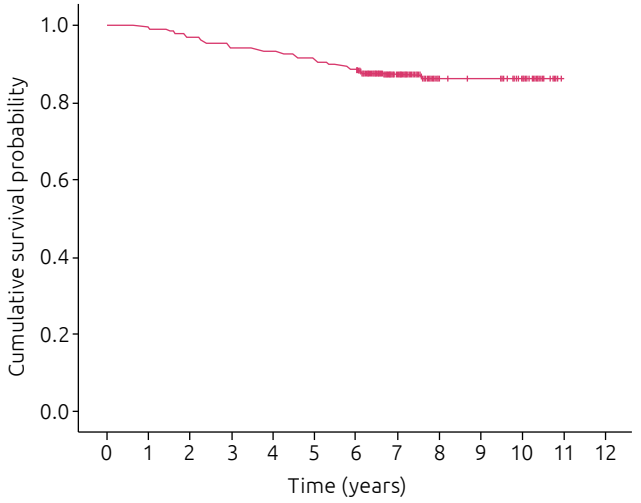
In total, the medical reports of 787 patients that comprised immunohistochemical and anatomopathological analyses of the mastectomy or quadrantectomy procedures were directly analyzed. After applying the eligibility criteria, the reports of 404 patients were eligible for the study. The mean age of the

Table 1. Characterization of the sample.

Variables	n=404
Age at diagnosis (years) – mean±SD	55.4±12.3
Current age (years) – mean±SD	61.8±12.6
Diagnosis – n (%)	
Infiltrating ductal carcinoma	326 (80.7)
Infiltrating lobular carcinoma	39 (9.7)
Infiltrating ductal and lobular carcinoma	8 (2.0)
Carcinoma *in situ*	31 (7.7)
Tumor size – n (%)	
Up to 2 cm in diameter	182 (45.0)
Between 2 and 5 cm in diameter	164 (40.6)
Over 5 cm in diameter	29 (7.2)
Any tumor size with chest wall or skin invasion	29 (7.2)
Histologic grade – n (%)	
G I	55 (13.6)
G II	204 (50.6)
G III	144 (35.7)
Lymph nodes – n (%)	
Lymph node metastasis (S)	133 (32.9)
No lymph node metastasis	271 (67.1)
Type of surgery – n (%)	
Quadrantectomy	284 (70.3)
Mastectomy	120 (29.7)
Skin invasion – n (%)	24 (5.9)
Nipple invasion – n (%)	15 (3.7)
Solitary nodule – n (%)	352 (87.1)
Presence of carcinomas *in situ* – n (%)	215 (53.2)
Tumor-free surgical margin – median (P25–P75)	0.3 (0.1–0.8)
Presence of inflammatory infiltrate – n (%)	136 (33.7)
Estrogen receptor – median (P25–P75)	90 (62.5–90)
Progesterone receptor – median (P25–P75)	40 (0–80)
HER2>30% – n (%)	50 (12.4)
Ki-67 – median (P25–P75)	10 (5–30)
Molecular classification – n (%)	
Luminal A	195 (48.3)
Luminal B	109 (27.0)
HER2	49 (12.1)
Triple negative	51 (12.6)
Death – n (%)	51 (12.6)
Recurrence – n (%)	94 (23.3)

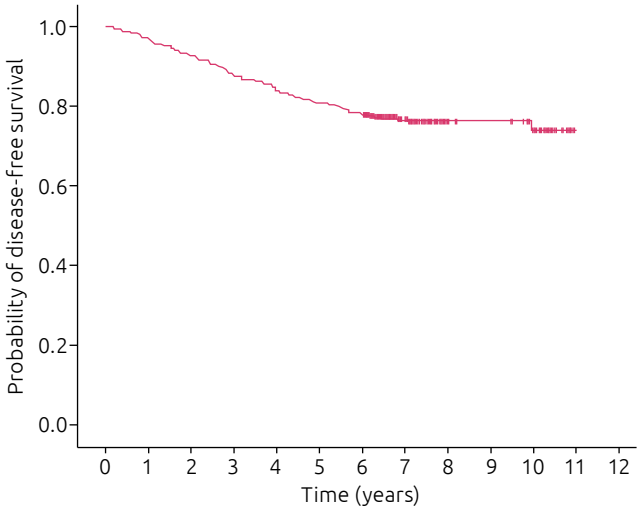
SD: standard deviation; HER2: human epidermal growth factor receptor type 2.

patients at the time of diagnosis was 55.4 years, with a standard deviation of 12.3. The mean age at the end of the analysis of the medical reports, on December 31st, 2018, was 61.8 years, with a standard deviation of 12.6. The diagnosis of greatest predominance was infiltrating ductal carcinoma, accounting for an 80.7% occurrence, followed by infiltrating lobular carcinoma, with 9.7%, and carcinoma *in situ*, with 7.7%. Taken together, the presence of ductal carcinoma and lobular carcinoma occurred in 2% of the sample.



Patients at risk	404	402	393	381	377	368	258	213	83	83	83	83
Survival rate (%)	100	99.5	97.3	94.3	93.3	91.1	88.6	87.4	86.4	86.4	86.4	86.4

Figure 1. Survival curve according to the Kaplan-Meier method.



Patients at risk	404	393	374	354	338	325	313	157	157	157	35	35
Event-free survival rate (%)	100	97.3	92.8	87.9	84.1	80.9	77.9	76.8	76.8	76.8	74.2	74.2

Figure 2. Disease-free survival curve according to the Kaplan-Meier method.

Variables with overall survival were associated with virtually all variables, except carcinomas *in situ*, tumor-free surgical margin, inflammatory infiltrate, and HER2. These same variables, in addition to the multinodal variable, were not significantly associated with disease-free survival.

To control confounding factors, the multivariate Cox regression model was performed (Table 3). After adjustment, current age, tumor size, lymph node metastasis, and Ki-67 remained associated with both overall survival and disease-free survival.

Molecular classification showed no significant relevance in the multivariate analysis.

The most frequent tumor size, according to the international classification system validated by the American Joint Committee on Cancer (AJCC) and by the Union for International Cancer Control (UICC), used as a tool in the staging of neoplasms, namely the TNM, was classified as T1, with tumors of up to 2 cm in diameter and occurrence of 45% in the analyses. Tumors between 2 and 5 cm in diameter, classified as T2, corresponded to 40.6% of the sample. Tumors classified as T3 and T4 stages corresponded to the remaining 14.4%. Among tumors classified as T4, the most present invasion was the skin one, with a 5.9% occurrence. Nipple invasion had a frequency of 3.7% of the sample.

According to the histologic grading modified by Elston and Ellis[22], the most frequent histologic grade was II, with 50.6%, corresponding to moderately differentiated tissues; followed by grade III, with badly differentiated tissues in 35.7% of the sample; and finally grade I, with well-differentiated tissues in 13.6% of the sample. Regarding lymph node involvement, 32.9% of patients presented lymph node metastases.

The use of neoadjuvant chemotherapy and the evolution of adequate staging and surgical techniques enabled to perform much more breast-conserving surgeries in the treatment of breast cancer. Thus, the most frequent surgical procedure in the study was the quadrantectomy, corresponding to 70.3% of the surgical profile identified in the sample. In this profile, the median of 0.3 cm of the surgical margin was maintained. A total of 53.2% of patients presented carcinoma *in situ*. Inflammatory infiltrate was present in 33.7% of the analyses. When there was presence of hormonal receptors, estrogen and progesterone, they represented a median of 90 and 40%, respectively. HER2≥30% occurred in 12.4% of the analyses. The Ki-67 proliferation index had a median of 10%.

The most frequent molecular classification was luminal A (48.3%), followed by luminal B (27%), HER2, and triple-negative (both with 12.6% each). The sample accounted for 12.6% of death and a total of 23.3% of recurrences.

DISCUSSION

As described in the literature[25], no statistically positive difference or evidence was found between the outcome of patients

who underwent quadrantectomy instead of mastectomy. In this sense, patients who underwent mastectomies had 2.06 times more deaths and 1.67 times more recurrences than patients treated with breast-conserving surgeries. Surgeries for the treatment of breast cancer have developed in such a way that major mutilating surgeries are being replaced with minimal surgical resections without impacts on the patients' prognosis[11].

Carcinoma *in situ* showed no statistical significance for the study, nor did the 33.7% of patients with inflammatory infiltrate.

In the univariate Cox regression analysis to evaluate factors, such as overall and disease-free survival rates, almost all factors were significantly associated. The mean age at the time of diagnosis was 55.4 years, which is similar to the mean of 56.8 years reported in other studies[8,9]. According to the regression analysis, age was associated with a 0.95 risk of death or recurrence. According to the univariate analysis, tumors classified as T2 increase the possibility of death by 2.31 times, and the possibility of recurrence by 1.7 times. Tumors with more than 5 cm in diameter, classified as T3, worsen the overall and

Table 2. Univariate Cox regression analysis to evaluate factors associated with overall survival and disease-free survival.

Variables	Overall survival		Disease-free survival	
	Hazard ratio (95%CI)	P	*Hazard ratio* (95%CI)	P
Age at diagnosis (years)	0.97 (0.95–0.99)	0.005	0.97 (0.95–0.99)	0.001
Current age (years)	0.95 (0.92–0.97)	<0.001	0.95 (0.92–0.97)	<0.001
Tumor size				
Up to 2 cm in diameter	1.00	–	1.00	–
Between 2 and 5 cm in diameter	2.31 (1.08–4.93)	0.031	1.70 (1.03–2.81)	0.038
Over 5 cm in diameter	6.61 (2.69–16.3)	<0.001	4.08 (2.10–7.96)	<0.001
Any tumor size with chest wall or skin invasion	9.56 (4.13–22.2)	<0.001	6.55 (3.58–11.9)	<0.001
Histologic grade				
G I / G II	1.00	–	1.00	–
G III	3.27 (1.85–5.78)	<0.001	2.11 (1.41–3.17)	<0.001
Lymph nodes				
Lymph node metastasis (S)	6.81 (3.63–12.8)	<0.001	3.67 (2.43–5.55)	<0.001
No lymph node metastasis	1.00	–	1.00	–
Type of surgery				
Quadrantectomy	1.00	–	1.00	–
Mastectomy	2.06 (1.19–3.57)	0.010	1.67 (1.10–2.53)	0.015
Skin invasion	5.38 (2.76–10.5)	<0.001	4.87 (2.83–8.36)	<0.001
Nipple invasion	5.11 (2.29–11.4)	<0.001	4.49 (2.33–8.68)	<0.001
Multinodular	1.97 (1.01–3.83)	0.047	1.39 (0.80–2.42)	0.242
Presence of carcinomas *in situ*	1.16 (0.66–2.01)	0.608	1.17 (0.78–1.76)	0.456
Tumor-free surgical margin	0.65 (0.34–1.25)	0.199	0.84 (0.54–1.32)	0.449
Presence of inflammatory infiltrate	1.17 (0.66–2.06)	0.590	1.29 (0.86–1.96)	0.221
Estrogen receptor	0.99 (0.98–0.99)	<0.001	0.99 (0.99–1.00)	0.001
Progesterone receptor	0.98 (0.97–0.99)	<0.001	0.99 (0.99–1.00)	0.011
HER2>30%	1.37 (0.64–2.91)	0.417	1.20 (0.67–2.16)	0.535
Ki-67	1.03 (1.02–1.04)	<0.001	1.02 (1.01–1.03)	<0.001
Molecular classification				
Luminal A	1.00	–	1.00	–
Luminal B	3.23 (1.54–6.79)	0.002	2.01 (1.23–3.26)	0.005
HER2	3.12 (1.26–7.76)	0.014	1.80 (0.95–3.43)	0.073
Triple negative	5.37 (2.41–11.9)	<0.001	2.26 (1.24–4.13)	0.008

95%CI: 95% confidence interval; HER2: human epidermal growth factor receptor type 2.

disease-free survival rates by 6.61 and 4.08 times, respectively, when compared to tumors smaller than 2 cm. Regarding T4 tumors, according to the univariate analysis, these tumors can worsen the overall and disease-free survival rates by 9.56 and 6.55 times, respectively. One fact that reinforces this statement is that skin invasion represented an increase of 5.38 times in the death rate and 4.87 times in the possibility of recurrence. Likewise, as T4 tumors, nipple invasion had a slightly more modest probability, with an increase in the possibility of death by 5.11 times and in the possibility of recurrence by 4.49 times. Tumor size compromises the favorable prognosis in larger lesions (>2 cm), mainly due to the impairment of more than 70% of the local lymphatic system[10,26,27].

The 1% increase in Ki-67 values raises, on average, by 2% and 1% the risk of death and recurrence, respectively. This factor is inversely proportional to the survival of patients with breast cancer[21]. The increase in Ki-67 is not only related to the proliferation of tumor cells, but also to the proliferation of blood vessels key to tumor growth and the metastasis process, since a neoplasm would not exceed 2–3 mm without a minimally adequate vascular network[10,28]. Tumor cell proliferation is related to prognosis in many tumors. The recognized aggressiveness of tumors classified as luminal B, when compared to luminal A ones, is probably related to Ki-67. It consists of a nuclear antigen present in the active phases of the entire cell cycle, with the exception of the G0 phase (resting phase). Although Ki-67 is essentially recognized for determining prognosis, it cannot be used as a basic criterion, since breast cancer is related to many factors that, together, determine the prognosis of each patient[20].

Only tumors classified as histologic grade III presented significant values of death or recurrence, accounting for 3.27 and 2.11 times, respectively, which occurs due to the ease of induction to post-chemotherapy cell apoptosis in breast cancer cells of histologic grades I and II[29].

According to the univariate analysis, the presence of lymph node metastasis increases death probability by 6.81 times and the risk of recurrence by 3.67 times.

Death probability was only statistically higher in triple-negative tumors, with a probability 5.37 times higher for death and 2.26 times higher for recurrence in patients within this classification. Although the triple-negative tumor, in many cases, presents a complete pathological response, this does not translate into better survival[20]. This finding corroborates the statement that triple-negative breast cancer has the worst prognosis, with disease-free survival between 14 and 17.8 months. Its guarded prognosis is closely related to the fact that this grade of breast neoplasia has no specific target therapy[30].

The luminal B subtype represented the second-worst prognosis in the univariate analysis, with a 3.23 times higher probability of death and a 2.01 times higher probability of recurrence when compared with luminal A — data that negatively outweigh even HER2 tumors, which presented overall survival 3.12 times worse and disease-free survival 1.80 times worse when compared to luminal A. The prognosis of HER2 tumors was better when compared to luminal B. This fact may be related to the treatment provided to HER2 patients, since HER2 tumors demonstrate

Table 3. Multivariate Cox regression analysis to evaluate factors associated with overall survival and disease-free survival.

Variables	Overall survival		Disease-free survival	
	Hazard ratio (95%CI)	P	*Hazard ratio* (95%CI)	P
Current age (years)	0.96 (0.94–0.98)	<0.001	0.96 (0.95–0.98)	<0.001
Tumor size				
Up to 2 cm in diameter	1.00	–	1.00	–
Between 2 and 5 cm in diameter	1.21 (0.54–2.69)	0.642	1.25 (0.74–2.10)	0.410
Over 5 cm in diameter	3.40 (1.32–8.75)	0.011	3.09 (1.53–6.23)	0.002
Any tumor size with chest wall or skin invasion	3.56 (1.41–8.99)	0.007	4.34 (2.25–8.36)	<0.001
Lymph nodes				
Lymph node metastasis (S)	4.11 (2.06–8.21)	<0.001	2.58 (1.64–4.08)	<0.001
No lymph node metastasis	1.00	–	1.00	–
Progesterone receptor	0.99 (0.98–1.00)	0.043	–	–
Ki-67	1.02 (1.01–1.03)	0.002	1.01 (1.00–1.02)	0.008
Molecular classification				
Luminal A	1.00		1.00	
Luminal B	0.90 (0.40–2.02)	0.793	0.81 (0.45–1.45)	0.478
HER2	1.20 (0.44–3.25)	0.722	1.06 (0.53–2.13)	0.865
Triple negative	1.24 (0.44–3.47)	0.679	1.08 (0.50–2.33)	0.843

95%CI: 95% confidence interval; HER2: human epidermal growth factor receptor type 2.

more satisfactory results when aggressive neoadjuvant treatments are administered, which benefit patients classified with this type of breast cancer[29].

Luminal A classification accounted for the best prognosis, which is probably related to the presence of the progesterone receptor. This receptor presented a positive relationship with a better prognosis, proving to be an independently associated factor, and its increase reduced the risk of death by 1%. This corroborates the results of recent studies whose authors report the association of prognoses significantly favorable to tumors with positive estrogen receptors[10,28,30].

In the multivariate analysis, no statistical relevance was found in the molecular classification.

Moreover, in this analysis, the one-year increase in age reduces the probability of death or recurrence, on average, by 4%. Death within a 10-year period is directly related to the presence of two factors: lymph node involvement and the age group of 60 years old or older[9].

Tumors of more than 5 cm in diameter and classified as T3, when analyzed in the multivariate analysis, increase the risk of death or recurrence by 3.5 times.

According to the same analysis, the presence of metastasis in lymph nodes increases the risk of death and recurrence by 4.78 and 2.63 times, respectively, differing from what is reported in the literature[10].

CONCLUSION

According to the molecular classification, among the predictive factors, the triple-negative tumor has the worst overall survival and the highest risk of recurrence, and luminal A classification presents the best survival. The increased presence of Ki-67 proved to be a reference factor for worse prognosis. Luminal B molecular classification accounted for the second worst prognosis, surpassing HER2 tumors. Among prognostic factors, tumor size, lymph node metastasis, and skin invasion were deemed reference factors for worse prognosis and lower overall and disease-free survival rates. Further studies and investigation of new markers are required in order to contribute to determining even more reliable prognoses.

AUTHORS' CONTRIBUTION

D. D.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Solfwares, Visualization, Writing – original draft, Writing – review and editing.

C. Z.: Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Supervision, Writing – review and editing.

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Histopathological and immunohistochemical parameters of breast cancer cases analyzed in a reference laboratory

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ABSTRACT

Objective: To determine the histopathological and immunohistochemical parameters of breast cancer cases treated in Belém, state of Pará, Brazil. **Method:** This is a cross-sectional, retrospective and observational study in which samples from 278 patients were analyzed. In the histopathological analysis were considered, among other factors, the differentiation and histopathological classification of the tumor, based on the WHO classification. As for immunohistochemistry, the presence and intensity of expression of the cell proliferation antigen Ki-67, gene product of HER2, and estrogen and progesterone receptors were evaluated. Then, the tumors were classified into luminal A, luminal B, luminal hybrid, HER2 group, and basal-like. **Results:** The most common histological subtypes were invasive carcinoma of no special type (88.7%), carcinoma *in situ* (5.5%), and invasive mucinous carcinoma (2.9%). The most common immunohistochemical subtypes were luminal A (26.1%), basal-like (23.6%), and luminal B (23.2%). We also found a statistically significant inversely proportional relationship ($p<0.01$) of hormone receptor expression with nuclear grade. **Conclusion:** The results show the importance of immunohistochemical analysis for staging, as well as for the therapeutic decision of each patient. However, further studies with a larger sample must be performed for more effective analysis of the general population.

KEYWORDS: breast cancer; immunohistochemistry; pathology.

INTRODUCTION

Breast cancer is a heterogeneous disease composed of multiple subgroups associated with distinct biological and histological characteristics, with different forms of clinical manifestation and patterns of response to current therapies. Histologically, invasive tumors are classified as invasive carcinoma of no special type (identified in medical practice as invasive ductal carcinoma — IDC), which corresponds to 70% of cases and is defined as a breast invasive epithelial neoplasm that does not meet the criteria for any special type, constituting a very heterogeneous group of tumors; and as the so-called histological special types, which are more homogeneous, with stricter diagnostic criteria, of which the invasive lobular carcinoma (ILC) is the most prevalent[1]. Histopathological parameters are traditionally used to evaluate tumor evolution by the Brazilian Society of Pathology (*Sociedade Brasileira de Patologia*).

Thus, the analysis of lesion size, axillary lymph node status, nuclear grade, and histological subtype are the basic aspects for defining primary prognostic factors. Histopathological characteristics of the lesion demonstrate different types of biological behavior of breast tumors[2].

However, the histological classification of breast cancer has weaknesses. In addition to the subjectivity of the diagnostic criteria, when applying such classification, about 85% of the cases end up belonging to the two main categories of IDC or ILC. Therefore, the system fails to group tumors with a broad biological spectrum and clinical behavior in the same categories, making histologic grading and the immunohistochemical evaluation of estrogen receptor (ER), progesterone receptor (PR), HER2, and the Ki-67 proliferation index to play a key role in increasing the discriminatory value among the different cases of breast carcinoma[3].

The presence of hormone receptors (HR) is associated with a more favorable prognosis. Therefore, patients with PR-positive tumors have longer disease-free survival and longer survival. Similarly, ER-positive tumors are associated with increased disease-free survival and also with a higher probability of response

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to hormone therapy. Conversely, patients with negativity for both receptors (ER and PR) showed worse prognosis than those with negativity for only one of the receptors[4].

Another important tumor marker is the HER2 proto-oncogene, which is responsible for the production of a protein that transmits signals for the growth of epithelial cells, whose expression is often increased in breast cancer. HER2 overexpression results in a more aggressive clinical behavior of the tumor, and the analysis of the marker status is an important factor in detecting types of cancer with a worse prognosis[5,6].

Tumors with high rates of cell proliferation are predominantly those with a high degree of malignancy. Thus, the evaluation of the mitotic activity is of paramount importance for assessing breast cancer. To that end, the cell proliferation index Ki-67 is used, a monoclonal antibody that detects a nuclear antigen, expressing cells entering the cell cycle and measuring the fraction of cell growth, thus enabling to detect tumors of a worse prognosis[5].

METHOD

Ethical aspects

Patients of the present research were studied according to the precepts of the Declaration of Helsinki and the Nuremberg Code, respecting the Ethical Standards for Research Involving Human Beings (Resolution No. 466/12), of the National Health Council. The investigation started after the submission and approval of the project by the Research Ethics Committee of *Universidade do Estado do Pará* and was authorized by the director in charge of the Paulo C. Azevedo Laboratory (*Laboratório Paulo C. Azevedo*) and the advisor responsible for the research.

Type of study, study population, and research site

This is a cross-sectional, retrospective, and observational study conducted at the Paulo C. Azevedo Laboratory, from March to June 2017. We evaluated medical reports of the histopathological and immunohistochemical examinations of breast tumors performed in the laboratory from January 2016 to January 2017. A sample of 278 patients was considered, whose size was calculated based on a universe of 1,000 patients.

In order to define this sample size, a formula was used to calculate samples with a universe of less than 100,000, according to Equation 1:

$$N = d^2.p.q.U / e^2 (U-1) + d^2.p.q \quad (1)$$

where the universe (U) of *y*, success rate of 50%, failure rate of 50%, standard deviation (d) of 2, and margin of error of 5% were adopted.

Inclusion and exclusion criteria

The sample included female patients over 18 years of age, whose medical reports of both histopathological and immunohistochemical examinations were stored in the archives of the Paulo C. Azevedo Laboratory, and who agreed to participate in the research by signing of the Informed Consent Form. All patients who presented only one of the required tests available and those who did not accept to participate in the study were excluded.

In the investigation protocol, the following data were collected: age, variables related to histopathological examination, and variables related to immunohistochemical examination.

Regarding histopathological aspects, the following were analyzed: tumor size; histologic/nuclear grade (differentiation grade); lymph nodes involvement and angiovascular invasion; presence of peritumoral inflammation; appropriate surgical margins; and histopathological classification of the tumor (IDC and ILC). As for immunohistochemical parameters, the following were evaluated: presence and intensity of expression of cell proliferation antigen (Ki-67); product of HER2 oncogene; and intensity of expression and presence of ER and PR (% percentage / + score).

After this evaluation, tumors were classified as: luminal A (ER+ and/or PR+ HER2 — and KI-67<14%); luminal B (ER+ and/or PR+ HER2 — and KI-67≥14%); luminal hybrid (ER+ and/or PR+ HER2+); HER2 group (ER-, PR- HER2+); and basal-like (triple-negative cancer ER-, PR- and HER2-).

Tumor size was classified into four types, according to the TNM classification updated by the American Joint Committee on Cancer[7]:

- T1: tumor size less than or equal to 2 cm in diameter;
- T2: tumor size greater than 2 cm, but less than or equal to 5 cm in its largest dimension;
- T3: tumor size greater than 5 cm in its largest dimension;
- T4: tumor of any size with extension to the chest wall or skin.

For the histological classification of invasive breast carcinoma, the World Health Organization (WHO)[8] proposal was considered, according to Table 1.

Data analysis

Data were structured in the Microsoft Office Excel 2007 program and analyzed through the IBM Statistical Package for the Social Sciences (SPSS) program, software version 17.0. Descriptive analysis of the number of cases of breast cancer was performed as well as that of absolute and relative frequencies of each subtype of immunohistochemical and histopathological classification. Descriptive statistics of the age of patients affected by cancer were performed considering mean, standard deviation, median, and minimum and maximum values, in addition to the representation of this variable by classification according to menopausal status (cut-off point=50 years of age).

Variables related to immunohistochemical analysis (ER, PR, product of HER2 oncogene, and cell proliferation antigen Ki-67) were cross-checked with the nuclear grade variable in order to verify correlations between them through Spearman's Correlation Coefficient, for ordinal variables, and Pearson's Correlation Coefficient, for scale variables.

Such immunohistochemical variables were also cross-checked with the presence of vascular invasion through the Mann-Whitney U test. The $p<0.05$ value was considered in all tests with the cut-off point for statistical significance.

DISCUSSION

Of the 278 cases of breast cancer analyzed at the laboratory in 2016, 26.1% were of the luminal A subtype; 23.6%, basal-like or triple-negative; and 23.2%, luminal B, as observed in Table 2. The results differ from those found by Cintra et al.[5], in whose study 41.8% of cases were classified as luminal B. However, the percentage of triple-negative subtypes was 24.2%, similar to that of the present study. Pérez-Rodríguez[9], in a study with 1,380 Mexican women, achieved similar results: luminal A was the most prevalent subtype, though with the most expressive percentage, of 65%, followed by the triple-negative (14%), and luminal B (12%). Mendoza del Solar et al.[10] found frequency of the triple-negative subtype in 30% of their sample, a number in line with our data. The triple-negative subtype is associated with more aggressiveness and worse survival[10].

It is worth highlighting a key point in the research conducted by Pérez-Rodríguez[9]: the luminal B subtype was classified according to the positivity of ER, PR, and HER2, which represents the luminal hybrid subtype of our study. This fact may explain the most expressive percentage of the luminal A subtype, since we considered cases with positivity for ER and PR in this subtype, and disregarded the percentage and the expression of the Ki-67 marker, which are generally used to distinguish luminal A and luminal B subtypes[11].

The fourth most frequent subtype was the luminal hybrid (13.8%) (ER+ and/or PR+ HER2+), a subtype poorly considered in similar research. The HER2+ subtype represented 10.1% of the cases analyzed in the period, a slightly higher value than the 8.92% perceived by Cherbal et al.[12] Southeast and South regions, with a higher percentage of European ancestry and higher socioeconomic status, tend to have a higher percentage of luminal tumors. The Northern Region presented more aggressive subtypes (HER2+ and triple-negative), whereas in the Midwest cases of triple-positive carcinomas prevailed. The Northeast, a region with a high percentage of African ancestry, presented intermediate frequency[13]. This observation by Carvalho et al.[13] may partly explain why, in the present study, lower percentages of luminal carcinomas and higher percentages

Table 1. Histological classification of invasive breast carcinoma.

Histological types
Invasive carcinoma of no special type
Invasive lobular carcinoma
Tubular carcinoma
Cribiform carcinoma
Carcinoma with medullary features
Metaplastic carcinoma
Carcinoma with apocrine differentiation
Adenoid cystic carcinoma
Mucoepidermoid carcinoma
Polymorphous adenocarcinoma
Mucinous carcinoma and signet ring cell carcinoma
Carcinoma with neuroendocrine features
Invasive papillary carcinoma
Invasive micropapillary carcinoma
Secretory carcinoma
Oncocytic carcinoma
Sebaceous carcinoma
Lipid-rich carcinoma
Glycogen-rich clear cell carcinoma
Acinar cell carcinoma

Source: WHO[8].

Table 2. Prevalence of breast cancer in a laboratory at Belém (PA), Brazil, in 2016, according to histopathological and immunohistochemical classifications.

Tumor subtypes	Frequency	
	N	%
Histopathological subtypes		
Squamous cell carcinoma	2	0.7
Carcinoma *in situ*	15	5.5
Signet ring cell carcinoma	1	0.4
Invasive carcinoma of no special type	244	88.7
Invasive lobular carcinoma	3	1.1
Invasive mucinous carcinoma	8	2.9
Invasive papillary carcinoma	2	0.7
Molecular subtypes		
Luminal A	72	26.1
Luminal B	64	23.2
Luminal hybrid	38	13.8
HER2	28	10.1
Basal-like	65	23.6
Unspecified	9	3.2

of triple-negative carcinomas were found when compared with those in the global literature.

Sánchez-Muñoz et al.[14], in a study with Spanish women, found a higher prevalence of luminal B subtype (51%), followed by luminal A (19%) and basal-like (5%) subtypes. Fourati et al.[15] identified a higher prevalence of luminal A (50.7%), followed by triple-negative (22.5%), and luminal B (13.4%) tumor subtypes. These variations are due to differences between the analyzed populations and also the use of different classification parameters, in addition to the immunohistochemistry itself[16].

The mean age at diagnosis was 53 years (±13.1), an age very similar to that surveyed by Pérez-Rodríguez[9], which was 53.3 years, and slightly below the mean of 57.5 years observed by Meattini et al.[17] However, the mean age observed by our study is slightly above that obtained by Cherbal et al.[12] These differences may occur due to the heterogeneous variety of women analyzed in these studies.

Regarding the histological classification of breast cancer cases, the most frequent type found in the present study was invasive carcinoma of no special type (88.7%), followed by carcinoma *in situ* (5.5%), and invasive mucinous carcinoma (2.9%). The frequency of invasive carcinomas of no special type in this study was higher than that identified by Caldarella et al.[18], of 58.5%. Meattini et al.[17] found IDC as the most common histological subtype (64%). Considering the new classification of invasive breast carcinomas according to the WHO[8], this subtype is included in the group of invasive carcinoma of no special type. The other histological types found were: ILC (1.4%), invasive papillary carcinoma (0.7%), and squamous cell carcinoma (0.7%). These data partly differ from the literature, especially when considering the low prevalence of ILC, which is generally responsible for 15% of breast cancer cases[8].

In a study conducted in Brazil, Smaniotto et al.[19] identified 70.49% of patients (n=86) with the IDC type. The second most frequent lesion was ILC, in 9.84% of cases (n=12). Furthermore, the authors pointed out 7.38% of cases of ductal carcinoma *in situ* (n=9). There was an incidence of 12.29% (n=15) for other types such as infiltrating ductal carcinoma, well-differentiated adenocarcinoma, invasive mucinous carcinoma, undifferentiated metaplastic carcinoma, and absence of carcinoma after neoadjuvant chemotherapy. These data partially corroborate the results of our study, especially when considering the high frequency of IDC; nevertheless, they differ regarding percentages of invasive lobular carcinoma and carcinoma *in situ*, which, in the first study, are higher.

According to Table 3, it can be observed that the expression of ER and PR was inversely proportional to the nuclear grade. Therefore, the highest expression of HR (ER and PR) was related to the lower nuclear grade. This inverse correlation proved to be statistically significant ($p<0.01$), similar to the findings of Dayal et al.[20], according to which when ER expression was null, the incidence of nuclear grade 3 was higher than 50%. Conversely, when the expression of ER was 3+, there was a higher incidence of nuclear grade 1. In a similar study conducted in Asia[21], ER positivity was observed in 70% of grade I carcinomas; in 48.2% of grade II; and in 3.5% of grade III ($p<0.001$). Likewise, PR positivity was perceived in 70% of grade I carcinomas; in 36.14% of grade II; and in 1.75% of grade III ($p<0.001$), which corroborates our results. Thus, we can perceive that better-differentiated tumors (lower nuclear grade) are more likely to be ER and PR positive, in addition to having a relatively better prognosis, since it is known that the presence of HR (ER and PR) in the tumor tissue is well correlated with the response to hormone therapy and chemotherapy[22].

On the other hand, we observed that the increased expression of Ki-67 was related to a higher incidence of high nuclear grade, since we found a positive and statistically significant correlation. This shows that high cell proliferation, demonstrated in the overexpression of Ki-67, is mainly present in carcinomas of higher histologic grade, being a marker of tumor progression and worse prognosis[23]. Such a result is in line with the findings of Narbe et al.[24], who also verified a significant positive correlation between Ki-67 and histologic grade ($p<0.001$), observing grade III tumors and Ki-67 mean value of 23.2%.

Moreover, Table 3 illustrates that HER2, although not statistically significant ($p>0.211$), presented the same trend as Ki-67 in relation to the histologic grade. A similar result was found by Arantes Júnior[25], who did not observe a statistically significant correlation, although he pointed out that the overexpression of HER2 was related to high nuclear grade (p-value ranging from 0.113 to 0.451). Thus, we found that the overexpression of HER2 seems to be an independent marker of biological aggressiveness, since it has no statistical significance when related to different levels of nuclear grade. Its overexpression in breast cancer indicates decreased survival due to poor prognosis and low response to tamoxifen (hormone therapy)[22].

Concerning tumor size, the mean size in patients with ER-positive tumors was 3.52 cm *versus* 3.73 cm in patients with ER-negative tumors, according to Table 4. Similarly, in patients with PR-positive tumors, the mean tumor size was 3.51 *versus* 3.72 cm in patients with PR-negative tumors; however, no significant correlation was established between tumor size and HR expression ($p=0.714$ and $p=0.698$, respectively). A similar result was found by Dayal et al.[20] and Ariga et al.[26]

It is known that lymph node status is important for determining breast cancer staging and treatment options. It is noteworthy that lymph node status consists of the most relevant factor in the prognosis of patients with breast cancer, since, as the number of positive axillary lymph nodes and the recurrence rate increase, the survival rate decreases. According to previous studies[20,27,28], there is a statistically significant correlation between HER2 expression and lymph node involvement and

vascular invasion, which has not been demonstrated for ER and PR. Nevertheless, this correlation was not found for any of these biomarkers in the present study.

CONCLUSION

Breast cancer is complex and heterogeneous, in addition to having a high prevalence in the female population. Hence, its correct classification is paramount for the best staging of the disease as well as for choosing the most appropriate therapeutic option. Therefore, immunohistochemical evaluation is key for the best diagnostic accuracy when associated with the tumor histopathological examination.

The present study aimed to evaluate the expression of ER and PR, the presence of HER2 oncogene, and proliferation antigen Ki-67, correlating them with the nuclear grade of the tumor. A higher prevalence of luminal A subtype was perceived, in addition to an inversely proportional relationship between the presence of HR and the nuclear grade of the tumor, with statistical relevance ($p<0.01$). Moreover, an important relationship was observed between the expression of the antigen Ki-67 and lower nuclear grade, i.e., with a lower differentiation grade and, consequently, worse prognosis.

Table 4. Distribution of the intensity of expression of hormone receptors according to tumor size.

Expression of hormone receptors	N	Tumor size		
		Mean ± standard deviation	Pearson's Correlation	p
Estrogen receptor				
Absent	96	3.79±3.03	-0.52	0.55
1+	27	3.87±2.68		
2+	32	3.55±2.20		
3+	120	3.47±3.01		
Progesterone receptor				
Absent	115	3.77±2.95	-0.61	0.49
1+	28	3.60±1.96		
2+	17	4.91±3.58		
3+	115	3.34±2.95		

Table 3. Correlation between intensity of expression of hormonal receptors, HER2 score, and Ki-67 product according to nuclear grade.

Expression intensity	Nuclear grade						Mean ± standard deviation	Spearman's Correlation Coefficient
	1		2		3			
	N	%	N	%	N	%		
Estrogen receptor								
Absent	0	0.0	41	54.7	34	45.3	2.45±0.50	-0.278*
1+	2	9.1	13	59.1	7	31.8	2.22±0.61	
2+	0	0.0	20	83.3	4	16.7	2.16±0.38	
3+	9	8.7	74	71.8	20	19.4	2.10±0.52	
Progesterone receptor								
Absent	1	1.1	51	55.4	40	43.5	2.42±0.51	-0.312*
1+	2	9.1	15	68.2	5	22.7	2.13±0.56	
2+	0	0.0	8	53.3	7	46.7	2.46±0.51	
3+	8	8.4	74	77.9	13	13.7	2.05±0.46	
HER2 Product								
Absent	4	4.7	56	65.9	25	29.4	2.24±0.53	0.084
1+	6	7.9	56	73.7	14	18.4	2.10±0.50	
2+	0	0.0	6	85.7	1	14.3	2.14±0.37	
3+	2	3.5	30	52.6	25	43.9	2.40±0.56	
Ki-67 product score								
[0.0–25.0%]	10	9.1	84	76,4	16	14.5	2.05±0.48	0.367*
[25.0–50.0%]	1	2.2	30	65.2	15	32.6	2.30±0.51	
[50.1–75%]	0	0.0	14	48.3	15	51.7	2.51±0.50	
>75.0%	0	0.0	19	50.0	19	50.0	2.50±0.50	

*Statistically significant difference ($p<0.01$) according to Spearman's Correlation Coefficient.

These results demonstrate the importance of tumor analysis performed according to immunohistochemistry and associated with histopathology. However, it is worth emphasizing that our research has limitations, especially due to the sample, and should be complemented with further studies addressing a larger number of patients.

AUTHORS' CONTRIBUTION

M.C.S.: wrote the original draft; I.J.M.R wrote the original draft.
I.C.T.S.A.: wrote the original draft.
J.V.P.A.: wrote the original draft.
A.M.M.: wrote the original draft.
L.B.D.J.: supervised and wrote the original draft.

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Factors related to non-mammographic visualization in locally advanced breast carcinoma

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ABSTRACT

Objective: To determine the rate and factors related to non-visualization of locally advanced breast cancer (LABC) by mammography. **Method:** Prospective, cross-sectional study, conducted in a cohort of consecutive patients with LABC treated at a tertiary cancer hospital. All patients were systematically examined and underwent high-resolution mammography (conventional equipment) in two views (craniocaudal and mediolateral oblique). A blind study was performed in which mammograms were mixed with routine and where radiologists were unaware of the clinical data. Three radiologists evaluated the examinations. In the patients in whom the findings were negative, the possible causes responsible for not identifying the tumor on mammography were evaluated. After the radiological report, the examinations were reviewed, and the radiological data were added to the standard form, making up the database of the present study. Descriptive statistics were used to compare factors related to non-visualization of tumors, namely the chi-square test and the Mann-Whitney test. **Result:** Eighty-five patients were evaluated. The average size of the tumors was 6.96 cm, and 20% of cases were not identified on mammography. Among the causes, 76.4% had dense parenchyma, 17.6% were not visible on examination, and in 5.8%, the lesion was not noticed by the radiologist (false negative examination). The only factor found when LABC was not identified was the type of breast parenchyma (p=0.04). **Conclusion:** Clinical history and changes in physical examination should be considered in the report to the radiologist. High breast density was the major obstacle to mammography diagnosis.

KEYWORDS: breast neoplasms; mammography; predictive value of tests; diagnostic errors.

INTRODUCTION

Mammography is one of the main radiological modalities for the diagnosis of breast lesions. It is related to the reduction of breast cancer mortality[1,2]. However, about 10 to 30% of breast cancers may not be diagnosed on mammography, the possible causes being: dense breast parenchyma, errors in perception, incorrect interpretation of suspicious findings, tenuous characteristics of malignancy and slow growth of a lesion[3-6].

In Brazil, there are several problems in mammographic screening, in which many patients, even if symptomatic, use mammographic screening campaigns of diagnostic task force to obtain diagnostic mammography.

Associated with this fact is that there is a delay in diagnosis along with the lack of appreciation of clinical complaints, and limitations of the health system, either because of the delay in mammographic results, associated with the quality of the mammography, or errors in the mammographic diagnosis process[7,8]. In patients who have gotten a mammogram properly, there can be issues such as interval tumors and the regular use of non-digital mammography[7]. Thus, many factors can lead to a negative finding, which can have medico-legal implications.

Locally advanced breast cancer (LABC) is still common in our country[7,9], mainly due to the lack of regular mammography, apart from difficulties in patient navigation to all diagnostic examinations[10].

There is a lack of studies that assess the percentage of lesions that are not identifiable by mammography. The identification of the factors associated with the non-visualization of tumors, even in LABC, is of utmost importance, aiming at a better understanding of the late diagnosis and the underestimation of potential radiological findings, justifying the present investigation.

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METHOD

We conducted a prospective, controlled study in patients with LABC, seen at a tertiary oncology hospital of the Unified Health System (SUS); the study was approved by the Research Ethics Committee No. 135/2008, which was registered at www.clinicaltrials.gov, NCT 00820690. Patients with non-metastatic LABC were evaluated. Data were collected from June 2008 to December 2009.

All patients with stage III breast cancer were submitted to a diagnostic delay questionnaire, systematically being directed to clinical examination, new mammography and breast ultrasound.

The inclusion criteria were:

- Patients with LABC, non-metastatic, stage III;
- Eastern Cooperative Oncology Group (ECOG scale) 0 or 1;
- Confirmed diagnosis of invasive ductal or lobular carcinoma.

The exclusion criteria were:

- Patients with extensive *peau d'orange*;
- Pregnant women;
- Primary inflammatory carcinoma;
- Ulcerated tumor;
- Failure to sign the informed consent form.

The patients underwent high-resolution mammography using computerized radiography equipment in two views (craniocaudal and mediolateral). The images were sent blindly and independently to three radiologists with extensive experience who were unaware of patient data and physical examination. In addition, these patients underwent ultrasound with dedicated high-frequency transducers; this was to assess the correlation between clinical examination and imaging examination. The density of the parenchyma was divided into four categories: breast almost entirely fat, breast with scattering of fibroglandular tissues, breast heterogeneously dense, and beast extremely dense; this is the new classification by the Breast Imaging-Reporting and Data System (BI-RADS). In patients with negative findings, the possible causes responsible for the failure to identify the tumor on mammography were evaluated. After the radiological report, and later, the data related to the radiological findings were added to the form, making up the database of the present study.

The data were recorded on a standard form and digitized for evaluation using the IBM Statistical Package for the Social Sciences (SPSS) for Mac, version 22. Descriptive statistics of the patients and mammographic findings are presented in Tables 1 and 2. We tried to group the main findings and compare them with non-identification in the mammographic examination, aiming to evaluate potential causes for the lack of identification of the lesion (Table 3). The χ^2 test was used to compare factors related to the non-visualization of tumors, and Fisher's test was used with values below 5. Continuous variables were assessed using the Mann-Whitney test. Values below 5% were considered significant.

RESULTS

Eighty-five patients, diagnosed with LABC, were evaluated. The main clinical findings are shown in Table 1. Mean age was 46.4 years (from 21.5 to 68.4 years). All patients were symptomatic and had a mean (± SD) complaint time and tumor size of 12.2±11.6 months and 6.9 ± 2.5 cm (2 to 15 cm), respectively. Of the total, 97.6% had unilateral involvement. Evaluating the clinical staging, 56.5% had stage IIIA, and 62.4% were T3, 72.9% N1 and 86.9% invasive ductal carcinoma.

Mammographic findings (Table 2) showed that 25.8% of patients had a dense or heterogeneous breast parenchyma. The main mammographic findings were the presence of a nodule (82.4%), microcalcifications (38.8%) and suspect lymph nodes (34.1%).

Of the patients, 81 (96.4%) underwent breast ultrasound. According to the echogenicity of the parenchyma, most were heterogeneous (45.7%), showing an irregular nodule (77.8%), with a hypoechoic pattern (93.8%) and shadow (61.7%) or posterior reinforcement (12.3%).

Of the lesions identified on physical examination, 20% (n=17) were not diagnosed on mammography (Table 1). Among the causes, 76.4% had dense parenchyma, 17.6% were not visible on examination, and in 6%, the lesion was not noticed by the radiologist (false negative). Figure 1 exemplifies a LABC case in which the tumor was not seen on mammography in a patient with a dense breast. Comparing the age group and the grouping of the main radiological findings, we found that the only and main factor associated with the non-identification of LABC was the type of breast parenchyma ($p = 0.04$; Table 3). Multivariate calculations were not performed because a single factor was identified with $p < 0.10$.

DISCUSSION

In general, the mammography examination in asymptomatic women is associated with a rate of non-visualization of lesions of around 10%. The findings of this study are noteworthy, in which 20% of symptomatic patients with confirmed biopsy had a normal mammography examination. This fact denotes the importance of the clinical data (asymptomatic/symptomatic) associated with the mammographic examination, as well as the inclusion of clinical information[8], since the radiological evaluation occurred blindly and since the radiologists were unaware of the patients' data.

There are barriers related to delayed diagnosis[11] relating to the health system, which can lead to an increase in the time between examinations; these can be problems related to the quality of radiological examinations, socioeconomic status, and distance from the referral service. In places where there is a limitation for the performance of a mammogram by SUS, in the presence of joint efforts or in opportunistic screening, the patient is able to get a radiological breast assessment, with the aim of reaching the referral service faster[8,12]. This fact is associated with problems in the patient's navigation, that is, in undergoing additional

tests until the definitive diagnosis of the neoplasm[13], which is common in our country, where patients take a long time from the onset of symptoms to diagnosis, often requiring additional tests and then being sent to the referral service for treatment[14]. Evaluating factors against the patient, there may be radiological characteristics that hinder the clear mammographic visualization of the lesion and tumor doubling time[15]. In this case series, only patients with LABC were included. Although LABC may be associated with smaller tumors, with extensive axillary involvement (N2/N3), this portion represented only 20% of the sample, and the tumor size and lymph node involvement were not associated with non-visualization.

Table 1. Clinical parameters and main mammographic findings.

Clinical finding	Parameter	Value (%)
Size	Mean (cm)	6.9±2.5
Age range	<40	25 (29.4)
	40 to 49	29 (34.1)
	≥50	31 (36.5)
Side	Right	29 (34)
	Left	56 (66)
Laterality	Unilateral	83 (97.6)
	Bilateral	2 (2.4)
T-TNM stage	T2	1 (1.2)
	T3	53 (62.4)
	T4	31 (36.5)
N-TNM stage	N0	6 (7.1)
	N1	62 (72.9)
	N2	14 (16.5)
	N3	3 (3.5)
TNM stage	IIIA	48 (56.5)
	IIIB	33 (38.8)
	IIIC	4 (4.7)
Histology	IDC	73 (86.9)
	ILC	5 (5.9)
	Others	7 (8.3)
Tumor in mammogram		
Size	Mean (cm)	6.2±1.9
Visualization	Two views	64 (75.3)
	One view	3 (3.5)
	Not visualized	17 (20)
Reason for non-visualizaton of tumors	Dense parenchyma	13 (76.4)
	Not visible on examination	3 (17.6)
	Lack of perception	1 (6)

TNM: TNM staging system; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma

The literature notes that mammography screening is performed in women over 40 years of age[2]. This study included women in a higher age group, but all had clinical evidence of a breast tumor, and the objective was to evaluate aspects associated with the non-visualization of tumors in the mammographic examination, demonstrating that breast density is an important factor, which is associated with age; however, age group was not seen to be an important factor here.

Several factors can influence non-visualization of tumors on mammography, and they can be grouped into four main ones[3-6]:

Table 2. Radiological mammography findings.

Radiological finding	Parameter	Value (%)
Parechyma	Lipo-substituted (0–25%)	30 (35.3)
	Partially lipo-substituted (25–50%)	33 (38.8)
	Heterogeneously dense (51–75%)	15 (17.6)
	Dense (>75%)	7 (8.2)
Skin	Normal	33 (38.8)
	Retracted	26 (30.6)
	Thickened	20 (23.5)
	Thickened + retracted	6 (7.1)
Nodule	Spiculated	27 (31.8)
	Irregular	24 (28.2)
	Lobulated	12 (14.1)
	No nodule	15 (17.6)
	Regular	7 (8.2)
Nodule border	Irregular	44 (51.8)
	Lobulated	25 (29.4)
	Not visible	14 (16.5)
	Regular	2 (2.4)
Microcalcifications	Absent	52 (61.2)
	Pleomorphic	11 (12.9)
	Other	22 (25.9)
Microcalcification distribution	Absent	52 (61.2)
	Grouped	19 (22.4)
	Segmented	9 (10.6)
	Ductal	5 (5.9)
Asymmetry	Absent	72 (84.7)
	Focal	9 (10.6)
	Diffuse	4 (4.7)
Lymph node	Not visualized	30 (35.3)
	Normal	26 (30.6)
	Dense	17 (20)
	Others	12 (14.1)

- patient (inherent or acquired dense breasts);
- tumor factors (minimal carcinoma, multifocal carcinoma and multicentric carcinoma);
- factors associated with the mammography technique (inadequate exposure factors, poorly positioned breasts and poor processing quality);

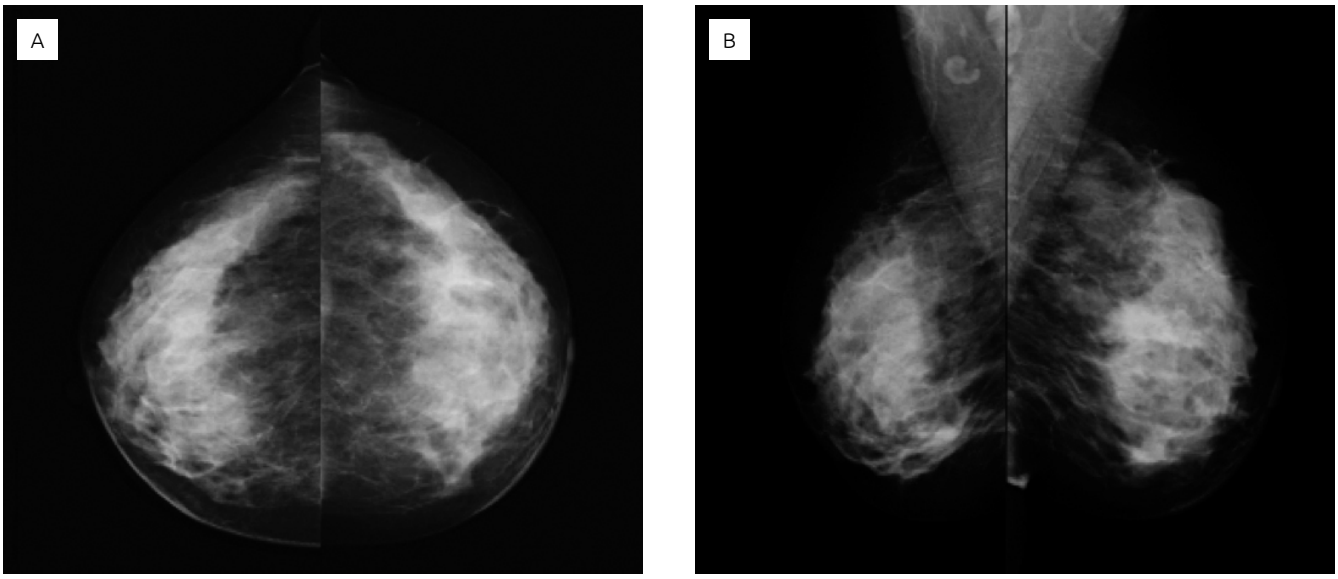

Figure 1. Mammography with no visible finding of tumor. Invasive ductal carcinoma in the left breast, T2N2M0 (stage IIIA).

Table 3. Factors related to non-identification of locally advanced breast cancer by mammography.

Category	Variable	Not identified n (%)	Identified n (%)	p
Clínical				
Size	Mean+SD	7.3±3.2	6.8±2.3	0.83
Age group	<40	5 (20)	20 (80)	0.74
	40 to 49	7 (24.1)	22 (75.9)	
	≥50	5 (16.1)	26 (83.9)	
Histology	IDC	16 (21.9)	57 (78.1)	0.46
	ILC	0	5 (100)	
	Others	1 (14.3)	6 (85.7)	
N-TNM	N0-1	13 (19.1)	55 (80.9)	0.74
	N2-3	4 (23.5)	13 (76.5)	
Mammography				
Parenchyma	0–25%	3 (10)	27 (90)	0.04
	51–75%	6 (40)	9 (60)	
	>75%	3 (42.9)	4 (57.1)	
Skin	Normal	5 (15.2)	28 (84.8)	0.42
	Anormal	12 (70.6)	40 (76.9)	
Nodule	No nodule	5 (33.3)	10 (66.7)	0.17
	Nodule	12 (17.1)	58 (82.9)	
Microcalcification	Absent	12 (23.1)	40 (76.9)	0.42
	Pathological	5 (15.2)	28 (80)	
Lymph node	Absent/not visualized	13 (23.2)	43 (76.8)	0.40
	Altered	4 (13.8)	25 (86.2)	

N-TNM: nodal TNM stage; SD: standard deviation; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma

- factors related to mammographic evaluation (poor perception and misinterpretation.

Even in the presence of negative radiological findings, mammographic screening is associated with the presence of interval tumors, which can be divided into true tumors, minimal findings and false negative tests (underestimation of radiological findings), making additional examinations and systematic clinical evaluation necessary, a fact that should determine the search for a professional, with the aim of repeating the examinations or combination of complementary examinations[16]. Microcalcifications and asymmetries can go unnoticed, needing attention[17].

Regular audits are needed to improve the technical quality of the radiological examination, minimizing potential causes of false negatives[18]. All patients, despite having undergone previous mammography, were systematically submitted to a new mammography examination at the service, which adheres to strict radiological quality programs, being accredited by the Brazilian Society of Radiology and, more recently, having undergone an international audit.

The type of equipment used can influence radiological findings, thereby interfering with the addition of radiological assessment software. Computer-aided detection (CAD)[19] raises sensitivity by 10%, for example. Mammographic screening studies were performed using conventional mammography, but digital mammography allows better visualization, although it has not been shown to be superior in mammographic screening[20]. Also, it decreases the incidence of interval tumors [21].

Two technologies are increasingly present in our daily lives: tomosynthesis[19], which improves sensitivity mainly in dense breasts; and spectral mammography, which increases sensitivity and specificity in relation to digital mammography (86.2–94.1% *versus* 53.4–85.9%)[22]. In this study, all mammograms were analog, and the examinations were evaluated by three radiologists with experience in mammographic screening, which enhances the importance of the findings presented here. Double-reading mammographic evaluation and evaluation by a senior radiologist decrease the rates of false negatives, compared to simple reading. Double-reading minimizes potential errors in perception and interpretation. In this sense, there is discussion regarding the possibility of simple reading with tomosynthesis[5], where the negative points would be the increase in radiation of the breast and the cost of the equipment.

Some radiological findings are associated with non-visualization of tumors on mammography, such as architectural distortion, asymmetries, unsuspected densities, anatomical location, lobular carcinoma, dense breast and lesion size[3,23]. In this study, the only factor that was associated with failure to identify the tumor was breast density.

Despite the small number of patients evaluated (n=85), we found a substantial number of mammograms with a negative finding (20%), even after evaluation by experienced radiologists and examinations performed under appropriate technical conditions, with internal clinical quality control, which denotes the importance of including and valuing clinical findings and the patient's clinical history.

Currently, when discussing mammographic screening, patients should be aware of the pros and cons of mammographic screening, but we must stress that it needs to be performed in asymptomatic patients. Clinical examination increases the detection rate[24], or minimizes negative radiological findings[25]. Symptomatic patients should seek out diagnostic services. Positive or doubtful clinical findings should warrant additional examinations, with ultrasound being an important complementary examination to be initially considered[6]. A study evaluating the potential reasons for non-visualization of tumors on mammography, given the identification of lesions by ultrasound, considered potential mammographic interpretation errors to be the presence of asymmetries, distortions and calcifications[18].

As limitations of the study, the radiological examinations were performed using conventional mammography, but nowadays in Brazil, most mammography uses this equipment, which reinforces our findings.

In the United States, radiology is the eighth specialty associated with medical procedures, and it is often related to problems of perception or interpretation[21]. The dissemination of knowledge about the limitations of mammography and the improvement of the doctor-patient relationship can minimize potential factors that can limit the radiological examination.

Mammography is one of the main tests related to the decrease in breast cancer mortality, a fact that should be valued. Increasingly, the patient must be aware of the pros and cons of mammographic screening and the limitations of mammography[1,2], in addition to the factors discussed in this article. Limitations should be part of the mammographic report, aiming at better knowledge on the part of the patient. Strict quality control, audited clinics and double reading can minimize the risk. This is associated with the presence of clinical history and clinical notes, which can influence the radiological report, and in the present study both were essential for the diagnosis of lesions not seen on mammography.

CONCLUSION

Rigorous observation after the mammographic examination, through clinical history, physical examination and image reading, must be considered in the radiological report, with the aim of reducing false negative rates. In this study, high breast density was the greatest obstacle, highlighting the importance of examining secondary aspects. The presence of asymmetries, distortions, changes in skin thickness and involvement of lymph

nodes is a warning sign that should be considered important, even in the case of no description of clinical findings.

AUTHORS' CONTRIBUTION

A.H.U.W.: conceptualization, data curation, formal analysis, funding investigation, methodology, project administration, supervision, validation.

M.M.S.: data curation, formal analysis, investigation, methodology.

B.E.F.C.: data curation, formal analysis, investigation, methodology.

R.A.C.V.: conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, resources, supervision.

All authors contribute to writing-original draft and performed writing-review & editing.

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REVIEW ARTICLE
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Prevalence and clinical implications of the TP53 p.R337H mutation in Brazilian breast cancer patients: a systematic literature review

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ABSTRACT

This study assessed the prevalence and clinical implications of the TP53 p.R337H mutation in Brazilian breast cancer patients through a systematic literature review. The literature review was performed in the PubMed, Scientific Electronic Library Online (SciELO), and Medical Literature Analysis and Retrieval System Online (MEDLINE) databases from 1997 to 2018. We used the keyword "R337H" in the search since it resulted in the largest number of published articles on the subject. Initially, we found 75 articles, and, after reviewing the titles and abstracts, we selected 18 studies investigating the prevalence of the TP53 p.R337H mutation in breast cancer patients and its clinical implications. The reading of the full texts led to the inclusion of seven studies. The studies were carried out in the states of São Paulo, Rio Grande do Sul, Rio de Janeiro, and Bahia. The TP53 p.R337H mutation was detected in 87 (4.8%) of the 1.789 women with breast cancer investigated. The prevalence of the TP53 p.R337H mutation in the selected studies ranged from 0.5% to 8.6%. These findings highlight the recommendation for screening the R337H variant in breast cancer patients in Brazil and suggest the need for new research addressing the clinical and prognostic aspects of breast cancer patients with TP53 p.R337H mutation-positive.

KEYWORDS: genes, P53; cancer; mutation.

INTRODUCTION

Breast cancer is an important public health problem, with high incidence in Brazil and worldwide. The study of breast carcinogenesis and risk factors for breast cancer is relevant to disease management, and numerous genes involved in the process of breast carcinogenesis have been identified.

Changes in the *TP53* pathway are significant in the pathogenesis of several human cancers[1]. In breast cancer, *TP53* mutations are found in 30%–35% of primary invasive tumors. However, the prevalence of mutations varies depending on the histological type of the disease, being found in up to 80% of triple-negative (TN) breast cancer, 10% of luminal A, 30% of luminal B, and in up to 70% of tumors rich in human epidermal growth factor receptor 2 (HER2)[2-4]. In Brazil, a *TP53* mutation called p.R337H draws the attention of professionals who deal with breast cancer, as it has been identified in a significant portion of patients with this type of cancer[5].

The tumor suppressor gene *TP53*, located on the short arm of chromosome 17 (17p13.1), encodes a nuclear phosphoprotein of 53 kilodaltons (kDa), which is responsible for regulating the expression of several genes that control the progression of the cell cycle, angiogenesis, and apoptosis, working as a transcription factor[6]. In normal cells, p53 is expressed at baseline levels. Nevertheless, when cells are exposed to agents that cause damage to the deoxyribonucleic acid (DNA), p53 expression increases and initiates transcriptional control of several target genes that prevent the cell cycle progression. Cell cycle

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blockage allows repair of cell damage, preventing replication of DNA lesions potentially involved in tumor induction, as well as the division of abnormal cells. In the case of extensive genomic involvement, p53 induces cell death due to apoptosis, preventing the spread of genetic changes[7].

Several functions are attributed to the p53 protein in the regulation of cellular response to genotoxic stress, such as that caused by ionizing radiation, free radicals, hypoxia, among others, as well as oncogene inactivation. The p53 protein also acts in the process of angiogenesis, cellular senescence, and inflammatory response[8]. The ability to recognize DNA damage and regulate the cell cycle closely connects the p53 protein to tumor suppression and cancer biology[9]. The p53 pathway can be influenced in several ways, either by the presence of somatic and germline mutations or by the presence of genetic polymorphisms. Several genes are involved in this cell regulation pathway, so a large spectrum of polymorphisms and mutations leads to individual variations in tumor phenotypes[9].

Mutations that change the function of the protein encoded by the *TP53* gene, preventing its tumor suppressor activity, are widely described[9]. One of them, called p.R337H, was first identified in Brazil among children with adrenocortical tumors in families without a family history of cancer[10]. The mutation located in exon 10 of the *TP53* gene, codon 337, consists of exchanging guanine (CGC) for adenine (CAC), which results in the replacement of the amino acid arginine (R) for histidine (H) at position 337 of the protein[11]. The mutated allele encodes a protein with changes in the C-terminal domain, producing unstable p53 tetramers, which compromise its tumor suppressor function[12]. The biochemical repercussion of this mutation affects the ability of p53 to form oligomers. The formation of oligomers depends on an optimal pH, and acid-base changes in the amino acid sequence of p53 affect its biochemical properties[12]. At pH 7, the ability to form oligomers does not change, but in a slightly basic medium, oligomer formation is impaired[13]. Given this theory, several phenotypic variations present in families carrying the *TP53* p.R337H mutation are described[14].

In Brazil, the *TP53* p.R337H mutation was initially detected in the Southern Region in individuals considered unrelated, but who later had their common ancestry elucidated[15]. The historical hypothesis explains the spread of the *TP53* p.R337H mutation by proposing that the opening of Estrada dos Tropeiros, a highway between São Paulo and the south of the country, led to the migration and distribution of *TP53* p.R337H carriers to the South and Southeast regions of Brazil, which characterized the so-called founder effect[16].

Some studies[17] have investigated the prevalence of the *TP53* p.R337H mutation in Brazilian women with breast cancer. However, when comparing the different regions of the country, there are variations in prevalence and a higher concentration of studies in the South and Southeast regions. The penetrance of the *TP53* p.R337H mutation is still poorly understood in Brazil, as well as its clinical implications in breast cancer. The *TP53* p.R337H mutation has proven to be relevant in the epidemiological context of cancer in Brazil, but few updated studies assess the prevalence and clinical implications of the mutation in the Brazilian population, especially for breast cancer[17]. Also, studies are concentrated in the South and Southeast of the country, while frequencies in other regions remain unknown.

This study comprises a systematic literature review that investigated the prevalence of the *TP53* p.R337H mutation in women with breast cancer in Brazil, as well as the association of the mutation with clinical implications of tumors. Given the relevance of the *TP53* p.R337H mutation in the current Brazilian scenario, this study can help oncology professionals in the clinical management of patients with the mutation and their families, as well as guide the development of new studies that address this issue.

METHODS

Search strategy

The bibliographic review was carried out in the PubMed, Scientific Electronic Library Online (SciELO), and Medical Literature Analysis and Retrieval System Online (MEDLINE) databases, from 1997 to 2018. We used the keyword "R337H" in the search, as it resulted in the largest number of published studies on the subject. The search was limited to articles published in Portuguese, English, and Spanish. Two researchers reviewed the titles and abstracts of the articles retrieved in the initial search to determine their relevance. Disagreements in the selection and inclusion of studies were solved by a meeting, re-reading, and discussion with a third researcher.

Eligibility criteria

The articles chosen were considered eligible when they met the following inclusion criteria:

- articles investigating the prevalence of the *TP53* p.R337H mutation in Brazilian women with breast cancer;
- articles studying the influence of the *TP53* p.R337H mutation as a marker in the prognosis of breast cancer patients with this alteration;
- studies associating the *TP53* p.R337H mutation with the risk of developing breast cancer;
- primary and descriptive studies;
- articles presenting a clearly described methodology;
- studies with consistent objectives regarding the methodology;
- articles in Portuguese, English, and Spanish fully available online.

According to the exclusion criteria, the following studies were not eligible:

- publications in languages other than Portuguese, English, and Spanish;
- studies with repeated cases;
- articles investigating other *TP53* mutations in Brazilian breast cancer patients;
- case reports and systematic literature reviews.

Data extraction and analysis

We extracted the following study data: title, first author, year of publication, study objective, population studied, number of participants, type of sample investigated, case origin, molecular methods of mutation assessment, and main results. The data obtained were reviewed and synthesized in tables.

RESULTS

Study selection

Initially, we found 75 studies by electronic data search. After reviewing the titles and abstracts of these articles, we selected 18 studies that investigated the prevalence of the *TP53* p.R337H mutation in breast cancer patients and its clinical implications. Reading the full texts of these articles resulted in the exclusion of 11 studies. In total, seven articles were eligible for the systematic review. Figure 1 shows the flowchart of the study selection process.

Characteristics of included studies

The seven studies included in this systematic review evaluated a total of 2,456 patients with and without breast cancer, with and without the *TP53* p.R337H mutation. The number of patients analyzed in the different studies ranged from 28 to 874, and the included studies were carried out in the states of São Paulo, Rio de Janeiro, Rio Grande do Sul, and Bahia. São Paulo and Rio Grande do Sul were the states that most researched the subject. The oldest article was published in 2008, and the newest is from 2014. All seven studies were published in English. Table 1 presents the characteristics of the studies included in the systematic review.

The mutation assessment methods in the selected studies included: polymerase chain reaction (PCR) associated with the analysis of restriction fragment length polymorphism (RFLP), comparative genomic hybridization based on microarrays (CGH-array), gene sequencing, high-resolution melting (HRM), immunohistochemistry (IHC), and real-time PCR (qPCR), using TaqMan probes. The study that used immunohistochemistry assessed p53 protein expression for the presence of the R337H mutation in tumor specimens. In general, the most adopted mutation analysis method was PCR-RFLP, in three studies, while the qPCR method was used in two studies, and gene sequencing was used to confirm the detected mutations.

All studies included in the analysis investigated the *TP53* p.R337H mutation in blood samples (Table 1), except one[18], which investigated the mutation only in specimens of phyllodes tumors. Two studies[19,20] that examined *TP53* p.R337H in blood samples also investigated the mutation in tumor samples.

Prevalence of *TP53* p.R337H mutation in Brazilian women with breast cancer

Seven studies investigated the prevalence of the *TP53* p.R337H mutation in a total of 1,789 women with breast cancer, of whom 87 (4.8%) had the *TP53* p.R337H mutation (Table 2). The frequencies of the *TP53* p.R337H mutation in the selected studies ranged from 0.5%[21] to 8.6%[20].

Among the selected studies, three were control cases[19,21,22], and they assessed the prevalence of the *TP53* p.R337H mutation in 1,208 women — 541 with breast cancer and 667 without breast cancer. The *TP53* p.R337H mutation was detected in seven of 541 patients in the case group (1.3%) and no woman in the control

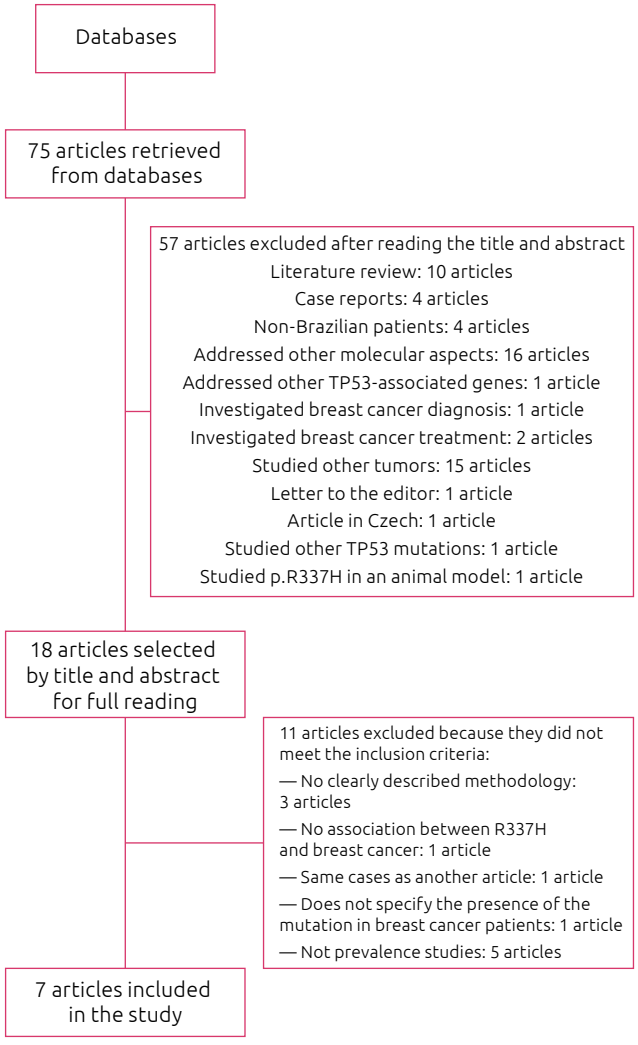

Figure 1. Flowchart of the study selection process.

Table 1. Characteristics of the studies included in the systematic review.

Reference	Case Origin	Objective/Sampling	Analyzed Biological Material/ Method	Results
Silva et al., 2014[14]	São Paulo, SP, Brazil	To investigate genetic changes in a group of 120 women with hereditary breast and ovarian cancer (HBOC) syndrome.	Blood. CGH-array and real-time PCR for mutation detection.	Three out of 120 women with breast cancer had the *TP53* p.R337H mutation.
Giacomazzi et al., 2013[18]	Porto Alegre, RS, Brazil; Barretos, SP, Brazil	To assess the presence of the *TP53* p.R337H mutation in 148 women with phyllodes tumor.	Tumor sample. Real-time PCR/ TaqMan and DNA sequencing.	Eight out of 148 women had the *TP53* p.R337H mutation, three with a malignant tumor and five with a benign tumor.
Assumpção et al., 2008[19]	Campinas, SP, Brazil	To determine the prevalence of the *TP53* p.R337H mutation in 123 women with breast cancer and 223 control women without breast cancer.	Blood and tumor sample. PCR-RFLP and IHC to detect the mutated protein.	Three out of 123 women with breast cancer had the *TP53* p.R337H mutation, and no women in the control group had the mutation.
Giacomazzi et al., 2014[20]	Porto Alegre, RS, Brazil	To assess the prevalence of the *TP53* p.R337H mutation in a group of 874 women with breast cancer.	Blood and tumor sample. Real-time PCR/TaqMan for mutation detection, DNA sequencing, and PCR-RFLP for tumor tissue analysis.	Out of the 874 breast cancer patients, 72 had the *TP53* p.R337H mutation.
Gomes et al., 2012[21]	Rio de Janeiro, RJ, Brazil	To assess the prevalence of the *TP53* p.R337H mutation in 390 women with breast cancer and 324 controls without breast cancer.	Blood. Allele-specific PCR (amplification refractory mutation system — ARMS) and DNA sequencing.	Two out of the 390 women in the case group had the *TP53* p.R337H mutation. No woman in the control group had the mutation.
Cury et al., 2014[22]	Ribeirão Preto, SP, Brazil	To investigate the prevalence of the *TP53* p.R337H mutation in 28 women with HBOC and 120 controls without cancer.	Blood. High resolution melting (HRM) for mutation detection.	Two out of 28 women with breast cancer had the *TP53* p.R337H mutation. No woman in the control group had the mutation.
Felix et al., 2014[24]	Salvador, BA, Brazil	To investigate mutations in 106 women with HBOC.	Blood. Allele-specific PCR, PCR-RFLP, and DNA sequencing.	One out of 106 women with HBOC had the *TP53* p.R337H mutation.

PCR: polymerase chain reaction; DNA: deoxyribonucleic acid; RFLP: restriction fragment length polymorphism; CGH-array: comparative genomic hybridization based on microarrays; IHC: immunohistochemistry.

Table 2. Studies that investigated the prevalence of the *TP53* p.R337H mutation in Brazilian patients with breast cancer (BC).

Reference	N	Inclusion criteria	Investigated gene region	Mutation screening method	N (%) p.R337H
Giacomazzi et al., 2014[20]	59	High-risk BC	*TP53* p.R337H	qPCR TaqMan, sequencing, and PCR-RFLP	2 (3.4)
Giacomazzi et al., 2014[20]	815	Unselected BC	*TP53* p.R337H	qPCR TaqMan, sequencing, and PCR-RFLP	70 (8.6)
Silva et al., 2014[14]	120	High risk BC	*TP53* p.R337H	CGH-array and qPCR	3 (2.5)
Giacomazzi et al., 2013[18]	148	Phyllodes tumor	*TP53* p.R337H	qPCR TaqMan, sequencing	3 (2.0)
Assumpção et al., 2008[19]	123	Unselected BC	*TP53* p.R337H, *TP53* geneexon 10	PCR-RFLP and IHC	3 (2.4)
Gomes et al., 2012[21]	390	Unselected BC	*TP53* p.R337H	ARMS-PCR, sequencing	2 (0.5)
Cury et al., 2014[22]	28	High risk BC	Full gene by HRM	HRM	2 (7.1)
Felix et al., 2014[24]	106	High risk BC	*TP53* p.R337H	AS-PCR, PCR-RFLP, sequencing	1 (0.9)

HRM: high-resolution melting; qPCR: real-time polymerase chain reaction; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; CGH-array: comparative genomic hybridization based on microarrays; AS-PCR: allele-specific PCR; ARMS: amplification refractory mutation system; IHC: immunohistochemistry.

group (Table 3). Two of these studies[19,21] reported that the women with breast cancer who had the *TP53* p.R337H mutation were under 45 years old. The third study[22] described two patients with *TP53* p.R337H, one diagnosed at the age of 30 and another with bilateral breast cancer, whose first cancer was detected at the age of 61, in the right breast, and the second at the age of 62, in the left breast. The data available in the selected studies did not allow a more detailed analysis of the age or clinical characteristics of patients with breast cancer and *TP53* p.R337H mutation.

Clinical implications in patients with the *TP53* p.R337H mutation and breast cancer

Information regarding clinical tumor characteristics, such as age at diagnosis, histological type, clinical staging, and status of immunohistochemical markers, is scarce in studies assessing the *TP53* p.R337H mutation in breast cancer patients. None of them followed the patients' response after the cancer diagnosis, nor did they assess the recurrence and/or survival of those carrying the *TP53* p.R337H mutation.

Regarding the age of the patients, a study carried out in Rio de Janeiro[21] evaluated a series of 390 breast cancer patients, with ages ranging from 25–60 years and a mean age of 46 years at diagnosis. Two patients (0.5%) under the age of 40 presented the *TP53* p.R337H mutation, one aged 35 years and the other aged 39 years. The two patients with the *TP53* p.R337H mutation reported a family history of other cancers.

The largest series of breast cancer cases selected in this review[20] investigated the prevalence of the mutation in women with breast cancer in different age groups. The study included 403 patients diagnosed with breast cancer before the age of 42 and 412 aged 55 years or older. The mean age of the patients at diagnosis was 38 (standard deviation — SD=5) and 66 (SD=9) years, respectively, in both groups. Invasive carcinomas were the most prevalent (90.5%), and the genotyping performed on tumor specimens showed a prevalence of the *TP53* p.R337H mutation of 8.6% in genotyped samples. The study also revealed an inverse relationship between age and mutation prevalence: in the group of women diagnosed at the age of 45 or younger, the prevalence was 12.1%, while in women diagnosed at the age of 55 or older, the prevalence was 5.1% (p<0.001). When women with breast cancer diagnosed at the age of 30 or younger were assessed, the prevalence of the mutation was 20% (8/40, 95% confidence interval — 95%CI 9.0–35.6%). The analysis of *TP53* p.R337H in the tumors indicated that, out of the 70 mutation-positive cases, 68 (97.1%) were heterozygous (c.1010 AG). Only two cases had mutant alleles detected in the tumors, suggesting that the patients were constitutive mutant homozygotes or hemizygotes.

Regarding the histological type of the tumors, most studies mentioned that the *TP53* p.R337H mutation-positive tumors were invasive carcinomas, without other specifications. One study[18] assessed the prevalence of the *TP53* p.R337H mutation in 148 women with phyllodes tumors, reporting the presence of the mutation in eight women and classifying the mutant cases as malignant (n=3), benign (n=5), and borderline (n=0). A malignant phyllodes tumor with the *TP53* p.R337H mutation has also been described in a study developed in the Southern region of the country[19].

DISCUSSION

In Southern Brazil, the germline *TP53* p.R337H mutation is highly associated with pediatric adrenocortical tumors and has low penetrance and limited tumor specificity in most families presenting this mutation. Among mutation-associated tumors, breast cancer is the most frequently found in *TP53* p.R337H-positive women, suggesting that this variant is relevant for breast carcinogenesis. Based on the studies included in this systematic review, the prevalence of the *TP53* p.R337H mutation in Brazilian breast cancer patients is high, ranging from 0.5% to 8.6%. These findings reinforce the recommendation for screening the R337H variant in breast cancer patients in Brazil.

The role of the R337H mutation in breast cancer is not yet clear. Most (90%) of the germline mutations in the *TP53* gene are in its DNA-binding domain. These mutations interrupt the protein structure and impair the function of the encoded protein. In contrast, the germline *TP53* p.R337H mutation occurs in the p53 tetramerization domain and seems to cause a more subtle

Table 3. Case-control studies that investigated the prevalence of the TP53 p.R337H mutation in breast cancer patients.

Reference	Type of study	Number of cases/ controls	TP53 p.R337H	Age of patients at diagnosis
Assumpção et al., 2008[19]	Control case	123 cases 223 controls	3/123 0/223	19 years, 29 years, and 44 years Mean age: 30.6 years
Gomes et al., 2012[21]	Control case	390 cases 324 controls	2/390 0/324	35 years and 39 years Mean age: 37 years
Cury et al., 2014[22]	Control case	28 cases 120 controls	2/28 0/120	30 years, 61 years (left breast), and 62 years (right breast) Mean age: 45.5 years

defect in the protein, which becomes functionally deficient only under certain conditions.

Germline *TP53* mutations are related to the Li-Fraumeni syndrome (LFS) with cancer predisposition. Individuals with germline *TP53* mutations have two characteristic disease phases, one in childhood with a tendency to develop rare cancers and one in adulthood with a tendency to develop more common cancers, but with early onset. The risk of childhood cancer versus adult cancer depends on the type of *TP53* mutation, as well as on genetic modifiers, including polymorphisms in *TP53* and genes encoding p53 regulators, such as murine double minute 2 (Mdm2), among others[9].

A recent study used a full genome sequencing to analyze a 2 Mb region at the *TP53* locus in samples of adrenocortical carcinomas. Selected common and rare variants were genotyped in 204 *TP53* p.R337H-positive cancer patients and a control group of 67,359 newborns. A commonly shared haplotype containing the E134* variant of the *XAF1* gene was detected in a subgroup (42%) of patients with adrenocortical carcinomas. This rare variant was identified in 70% of patients with *TP53* p.R337H. The cosegregation of both variants was found in 79% of cancer patients and was significantly higher in individuals with sarcoma and multiple malignancies, including breast cancer[23]. The results of this study should be expanded and may contribute to elucidate the role of the *TP53* R337H mutation and its modifiers.

The studies included in this review were conducted in the states of São Paulo, Rio de Janeiro, Rio Grande do Sul, and Bahia. São Paulo and Rio Grande do Sul had the largest number of publications on the subject, and the highest prevalence of *TP53* p.R337H mutation in women with breast cancer was found in Porto Alegre (8.6%) and Ribeirão Preto (7.1 %). A study carried out in Bahia showed that one out of 106 women with breast cancer assessed had the *TP53* p.R337H mutation, indicating that the mutation is not restricted to the South and Southeast regions[24].

One of the studies included in the systematic review[20] investigated the prevalence of the *TP53* p.R337H mutation in a large group of breast cancer patients from three important reference centers for cancer treatment in Brazil and performed the geographical distribution of the cases assessed. The study revealed a significant variation in the disposition of breast cancer cases with the *TP53* p.R337H mutation. This variation can be explained by the differential dissemination of the founder haplotype in some regions of the country due to the migratory effect and sociodemographic differences that intrinsically affect the risk of developing breast cancer in the Brazilian population. The lack of studies in different geographic regions of Brazil demands the development of new research on this subject.

The studies included in this article used several methods to detect the *TP53* p.R337H mutation, especially PCR-RFLP and qPCR with TaqMan probes. An investigation that assessed 95 genomic DNA samples compared the performance, cost, and response time of the Sanger, PCR-RFLP, TaqMan-PCR, and HRM sequencing methods employed in the *TP53* p.R337H genotyping, and the results were 100% concordant for all methods[25]. Nonetheless, DNA sequencing is considered the gold standard among the methods and recommended to confirm the mutation.

This systematic review included three case-control studies[19,21,22]. The *TP53* p.R337H mutation was detected in seven of the 541 patients in the case group (1.3%), and none of the 667 women in the control group. Despite the considerable number of cases evaluated, the heterogeneity of the studies did not allow a combined analysis of the data in the form of meta-analysis, which prevented the assessment of the risk of *TP53* p.R337H-positive patients developing breast cancer.

An important limitation of this study is the fact that prognostic aspects of *TP53* p.R337H-positive breast cancer could not be assessed since none of the included articles addressed these variables. Retrospective studies that include large series and the possibility of patient follow-up are necessary to elucidate the prognostic role of the *TP53* p.R337H mutation in breast cancer.

As described in the "Results" section, information regarding clinical tumor characteristics, such as their histological type, clinical staging, and status of immunohistochemical markers, was extremely scarce in the studies included in this work. Immunohistochemical data from 66 breast cancer patients positive for *TP53* p.R337H were reviewed and compared to data from 12 patients with other functional *TP53* mutations[26]. In the group of patients with other functional *TP53* mutations, 75% of the tumors showed overexpression of HER2 (3+), corroborating previous studies, while 22.7% of the patients with *TP53* p.R337H presented HER2 overexpression. These results reinforce the hypothesis that different germline *TP53* mutations act through different pathways of carcinogenesis, suggesting that the histopathological and immunohistochemical aspects of *TP53* p.R337H-positive breast cancer should be further investigated in future studies.

The seven studies included in this review showed that 87 (4.8%) of the 1,789 women with breast cancer investigated in Brazil had the *TP53* p.R337H mutation. These results indicate that the *TP53* p.R337H variant contributes to an important portion of breast cancers diagnosed in our population and that screening for this variant needs to be considered in the diagnosis and prevention of these tumors. The prevalence of the *TP53* p.R337H variant is high when compared to other particular mutations detected in *TP53* and should be taken into account in the genetic counseling of Brazilian breast cancer patients.

AUTHORS' CONTRIBUTIONS

V.A.S.: Conceptualization, funding acquisition, investigation, methodology, investigation, project administration, supervision, validation, visualization, writing – review & editing.
D.C.A.: investigation, validation, visualization, writing – review & editing.

E.S.V.C.: Data curation, formal analysis, Investigation, writing – original draft.
I.F.M.: Data curation, formal analysis, investigation, writing – original draft.
N.A.N.: Conceptualization, data curation, formal analysis, investigation, visualization, writing – original draft, writing – review & editing.
F.M.A.: Methodology, validation, writing – review & editing.

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REVIEW ARTICLE
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Robotic breast surgery: the pursue for excellence in treatment and satisfaction – a review

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ABSTRACT

Introduction: Nipple sparing mastectomy (NSM) with immediate reconstruction is an option for the treatment of breast cancer or for risk-reducing surgery. This technique offers good aesthetic results without compromising oncological safety. Robotic nipple sparing mastectomy (RNSM) was first described in 2015 and has been executed in various centers ever since, but the cost-effectiveness and oncological safety of this technique are still questioned. **Objectives:** The primary aim of this study was to critically review the literature and discuss the feasibility, advantages and limitations of robotic breast surgery. **Methods:** Search in PubMed database for publications related to "robotic breast surgery". Selection and review of relevant articles, and analysis of results from these studies. **Results:** Our search comprised the period between 2015 and 2019. The rates of complications were low and the learning curve is apparently rapid, though there is still a lack of data involving cost-effectiveness. **Conclusions:** RNSM with immediate reconstruction is a great advance in the surgical treatment for breast cancer. Cost-effectiveness and oncological safety must still be accessed through randomized clinical trials.

KEYWORDS: breast neoplasms; robotic surgical procedures; mastectomy, subcutaneous; breast implants.

INTRODUCTION

Breast cancer diagnosis and surgery have evolved toward less invasive procedures throughout the years. Breast conserving surgeries are largely carried out and mastectomies no longer have to be disfiguring. More than ever, breast surgeons are committed to improve their techniques in order to offer better aesthetic outcomes, which relate to better quality of life and self-image appreciation[1].

Nipple sparing mastectomy (NSM) was described in 1984 by Hinton et al. as a safe alternative to simple mastectomy. In a series of 98 patients submitted to subcutaneous mastectomy, the skin envelope was preserved and reconstruction was performed about 6 months later; there was no increase in local recurrence of the skin flaps in a follow-up of 30 months[2]. The term NSM with immediate reconstruction was first used by Toth and Lappert in 1991, and in the same year by Kroll et al., who published a series of 104 cases, with similar local recurrences, after a mean follow-up of 5.6 years[3,4]. NSM is nowadays an option for the treatment of breast cancer, when following appropriate indications, and also for risk-reducing surgery, offering good aesthetic results without compromising oncological safety[5].

More recently, endoscopic breast surgery was attempted, but due to technical difficulties, it was not adopted in clinical practice[6,7]. In the context of minimally invasive approaches, the use of robotic surgery has become popular in urologic, gynecological, and colorectal procedures, and more recently, in the fields of thyroidectomy, oropharyngeal, and plastic surgery[7]. The first report of breast robotic surgery happened in 2015 by Toesca et al., who performed robotic nipple sparing mastectomy (RNSM)[8] with a DaVinci S robotic platform and since then a similar procedure has been executed in other centers. Surgeons claim that the advantages of RNSM are better aesthetic outcomes, with minimal scars hidden under the arm, enhanced precision with three-dimensional optics, reduced tremor and less bleeding[7-10]. The objective of this review was to discuss the feasibility, advantages, and limitations of robotic breast surgery, especially RNSM.

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METHODS

A search was performed in PubMed database for articles related to robotic breast surgery, published from 2015, year known to be the first report, until June 2019. The search identified 163 related articles. Titles that did not relate to breast surgery or breast cancer were excluded. This resulted in 27 abstracts to be read, which mentioned internal mammary robotic surgery, robotic harvesting of flaps, or RNSM with or without robotic reconstruction. Only the 19 abstracts mentioning RNSM were considered and read in their entirety. Of these, six were selected to analyze the data, excluding duplicates, editorials, letters to the editor, or response to letters to the editor. Surgeries performed in cadavers were not included in the data analysis, but considered for technical detail information.

RESULTS

The first report of RNSM was carried out in 2015 by Toesca in the Istituto Europeo di Oncologia (IEO), with the objective to study an innovative technique and overcome the limitations of the endoscopic approach. Three patients with BRCA mutations, previously treated for unilateral breast cancer, who wanted to undergo a contralateral risk-reducing surgery were submitted to the procedure[8]. Following this, Sarfati et al. conducted a similar procedure on breasts of two fresh female cadavers[9].

Since then, other centers have published their cases, describing different aspects in positioning, incision, complications, and follow-up results. Studies data are summarized in Table 1.

Patients

The studies involve a total of 160 patients. Toesca et al.[7] reported that their first three cases were prophylactic contralateral RNSM in patients previously treated for breast cancer, but after they gained knowledge of how to remove the gland, they extended the indication for patients with breast cancer, reporting a total of 29 RNSM in 24 women. The tumor had to be situated at least 1cm from the nipple areola complex (NAC), in patients with no associated comorbidities, body mass index (BMI) < 25, and who were at low risk for anesthesia. Exclusion criteria were: grade 2 ptosis or higher, diabetes, heavy smoking, obesity or previous radiation therapy. In 2016, Sarfati et al. reported their first experience with RNSM in two fresh female cadavers[11], and later in June 2018, published their study involving 62 prophylactic, and only 1 therapeutic RNSM[9]. The breasts had ptosis grade 1 or 2, they were of small breast cup size, the tumor had to be at least 2 cm away from the NAC, and a high-risk genetic mutation had been identified in the prophylactic group. Patients were excluded if they had a history of breast surgery or radiation, if post-operative radiation was required, and also heavy smokers or patients with uncontrolled diabetes mellitus. Lai et al.[10] performed 39 RNSM in 33 women, most of which (35 breasts) were therapeutic. Patients were diagnosed with ductal carcinoma *in situ* (DCIS) or invasive breast cancer stages I, II, or IIIA, with a tumor size < 5cm and no evidence of multiple lymph node metastasis. Patients with severe comorbidities, skin, chest or nipple invasion, locally advanced or inflammatory disease were excluded. Houvenaeghel et al.[12] performed 27 RNSM in 17 patients with primary breast cancer and 10 with local recurrences. Characteristics of patients were determined and they were divided into three groups, each with different approaches for breast dissection. Park et al.[13] and Rajappa et al.[14] describe each, their experience with 1 case only.

Positioning

Toesca et al. first described a flat supine position, with the arm above the head, internal rotation, and 90º abduction, lying on a chopping block placed under the back[8], but this patient developed a temporary biceps brachii strength reduction. Because of that, in the following cases, the upper arm hung normally alongside the body, and the elbow was bent at about 30º so that the hand, wrist, and forearm were straight and roughly parallel to the floor at the side of the bed[7]. Sarfati and Lai describe a supine position with abduction at 90º of the arm[9,10]. Houvenaeghel et al. and Park et al. describe a supine, dorsal decubitus, with ante-flexion of the arm[12,13]. Rajappa et al. reported positioning as Toesca's et al.[14].

Incision and technique

Different techniques were described, though having one thing in common: an incision under the axilla, hidden by the arm. Incision size varied from as small as 2.5 to 6cm, in the mid-axillary or anterior axillary line. This size is mainly determined by the size of the breast to be removed through the same incision. In some series, a second small incision was made inferior to the first, in order to insert another trocar and the drain at the end of the procedure[9,12]. Most studies describe subcutaneous flap dissection with non-robotic scissors or electrocautery[7,9,13,14] to gain space for placing the port and docking. Houvenaeghel et al.[12] divided their patients into three groups in order to compare time of procedures:

- group 1: dissection with robotic scissors using coagulation;
- group 2: dissection with robotic scissors without coagulation;
- group 3: dissection with non-robotic scissors after subcutaneous infiltration with adrenaline serum and then robotic dissection.

Except for Park et al.[13], who used no gas but retractors to maintain the working space, all other surgeries were performed under low pressure of 7-8 mmHg of carbon dioxide[7,9,10,12,14]. Dissection of the gland was performed with monopolar curved-scissors or cautery, moving from the axilla toward the nipple areola complex, medially, superiorly and inferiorly around the breast. An intraoperative biopsy of the retroareolar region in therapeutic surgeries was usually done with intraoperative frozen sections in series by Toesca et al. and Park et al. Lymph node dissection was performed through axillary incision, so as the removal of breast

gland, placement of prosthesis and, in cases of reconstruction with the latissimus dorsi, dissection of the flap were also done through the same incision.

Surgery time

It is understandable that with a new technique, surgical time will be long. The first operation by Toesca et al. took 7 hours, needing conversion to open surgery, due to prolonged surgery time[8]. The last cases were completed in about 3 hours, including docking, dissection and reconstruction. All studies report the same outline, with a fast learning curve. In Houvenaeghel et al.'s study, the different groups had very different surgery times, and the longest procedures were those with robotic dissection[12]. According to Lai et al., the larger the breast, the longer time was needed in the initial cases, but operation time decreased significantly in the mature phase and did not fluctuate with specimen weight[10]. Another factor that has influence over surgical time is the prophylactic or therapeutic indication of procedure, because of the need to do a biopsy of retroareolar region, with intraoperative frozen section. Surgical time data can also be visualized in Table 1.

Complications

The rate of complications or conversions in the studies was low, most of them classified as minor complications, grade I, II or III, according to the Clavien-Dindo classification[15] (Figure 1). Erythema was described in one patient; small blistering of the skin, caused by electrocautery was reported in four patients. Seroma needing aspiration in one patient; dorsal lymphocele in one patient; and hematoma needing operation in one patient. Neuropraxia happened in two cases, both temporary. One axillary delayed wound healing was reported. There was partial nipple ischemia in four patients, partial skin flap (not

Table 1. Summary of studies data.

Study	Patients	Positioning	Incision	Surgery Time	Oncological Outcomes	Satisfaction	Cost-effectiveness
Toesca et al.[7]	24 patients - 29 breasts: 21 therapeutic; 8 prophylactic RNSM	Flat supine position; arm alongside the body	3 cm on midaxillary line	420 min (first case); 180min (last cases)	No recurrence. 8 months follow-up	High degree*	N/A
Sarfati et al.[9]	33 patients - 63 breasts; 1 therapeutic; 62 prophylactic RNSM	Supine; 90° abduction of the arm	Vertical 3–5 cm + a subcentimeter incision 8-9 cm below, 6–7 cm posterior from the lateral-mammary fold	195 min (first case); 85 min (last cases)	No recurrence. 9 months follow-up	Evaluation in progress	N/A. Reduction of operating time may overcome the issue of operating room efficiency
Lai et al.[10]	33 patients - 39 breasts; 35 therapeutic RNSM	Supine; 90° abduction of the arm	2.5-5 cm oblique axillary incision	287.2 ± 77.43 min (cases 1-13); 235.6 ± 30.69 min (cases 14-39)	No recurrence. Mean 8.6 ± 4.5 months follow-up	N/A	N/A
Houvenaeghel et al.[12]	27 patients - 27 breasts; 27 therapeutic RNSM	Supine, dorsal decubitus, with anteflexion of the arm	Vertical 4-6 cm; on anterior axillary line + incision for trocar inferiorly	372.5 (group 1) 303.4 (group 2) 257.7 (group 3)	N/A	N/A	N/A. Fixed costs and cost of robotic instruments can provide more costs than conventional surgery
Park et al.[13]	1 patient. Therapeutic RNSM	Supine, dorsal decubitus, with anteflexion of the arm	Vertical 6 cm; on anterior axillary line	409 min	No recurrence. 12 months follow-up	N/A	N/A
Rajappa et al.[14]	1 patient. Therapeutic RNSM	Flat supine position; arm at the side of the body	3 cm on midaxillary line	330 min	N/A	N/A	N/A

RNSM: robotic nipple sparing mastectomy; N/A: Not applicable
Summary of technique, oncological outcomes, patient satisfaction and cost effectiveness in the studies analyzed. * Satisfaction described in study, but no satisfaction questionnaire cited.

involving the nipple) in three patients, and no cases of total NAC necrosis. Infection was reported in three patients, two of which needed revision, resulting in one implant loss in one series[9]. In another, reoperation was necessary for four patients, with three cases of prosthesis explantation[12]. Conversion to open surgery occurred in four cases, due to bleeding of internal mammary perforator (2 patients), malpositioning of incision causing technical problems (1 patient), and in Toesca et al.'s first case, due to long time of surgery (1 patient). Implant rotation was reported for 1 patient, and there was no information on whether the patient was reoperated. Complication events are summarized in Figure 2.

Oncological outcomes

There were no recurrences in the studies analyzed, with the longer follow-ups in Park et al.'s case report — 12 months —, and in Sarfati et al.'s series of cases — 9 months[9,13].

Satisfaction

Despite the surgery's cost and time, the satisfaction of the patient must be evaluated to determine advantages of robotic procedures. None of the studies have objective satisfaction rates published. Toesca et al. describe patient satisfaction as "high degree", but no questionnaires were used[7]. Sarfati et al. used the Breast-Q questionnaire before the procedure, another non-specified satisfaction questionnaire at 6 months, assessing amongst other things the aesthetic result, and the Breast-Q and the satisfaction questionnaire were planned to be used again at 12 months[9]. Data are not yet available.

Cost-effectiveness

Robotic surgery is usually considered a very expensive procedure because of fixed and of robotic instruments costs[12]. The studies analyzed do not assess cost-effectiveness of RNSM.

DISCUSSION

In an era were minimal invasive techniques arise and gain popularity, robotic surgery emerges with the proposal of delivering excellence in oncological treatment at the same time as it provides good aesthetic results. According to these recent studies, with short follow-ups, indeed this technique seems to meet its promise.

The question is if it is really worth the price[16]. Robotics is known for its high costs, related initially to the purchase of the da Vinci Surgical System that costs between US$1 and US$2.3 million, added to maintenance fees, from US$100,000.00 to US$150,000.00 annually. The instrument arms of the robot have a maximum of 10 uses, after which they can no longer be used[17]. Moreover, robotics demands adequate staff training, infrastructure upgrades, and increased operating room time. These costs are, in some cases, offset by shorter hospital stays, less trauma, bleeding and operative complications[18,19].

In the context of breast surgery, bleeding is not a major problem and patients usually are discharged from hospital in a few days. NSM with immediate breast reconstruction, either with prosthesis or a flap, is one of the largest breast procedures, and for this reason, robotic surgery may be a good alternative.

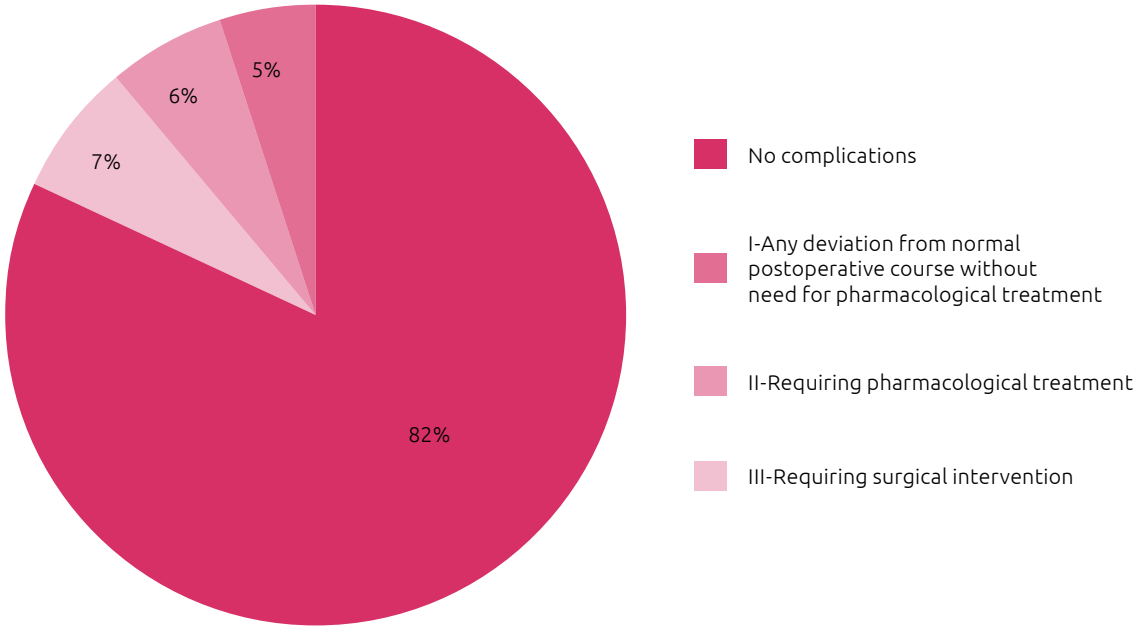

Figure 1. Classification of complications in robotic nipple sparing mastectomy, according to Clavien-Dindo grade.

Centers worldwide are studying its safety and feasibility and data on its cost-effectiveness are soon expected.

Earlier this year, Linhares et al. performed the first breast robotic surgery in Brazil at Erasto Gaertner Hospital[20]. Other cases have followed and we soon expect a national publication of their experience.

CONCLUSIONS

RNSM with immediate reconstruction with breast implant is apparently a safe approach to the removal of the breast gland, but studies have short follow-ups of only a few months. Longer follow-up is necessary to prove oncological safety.

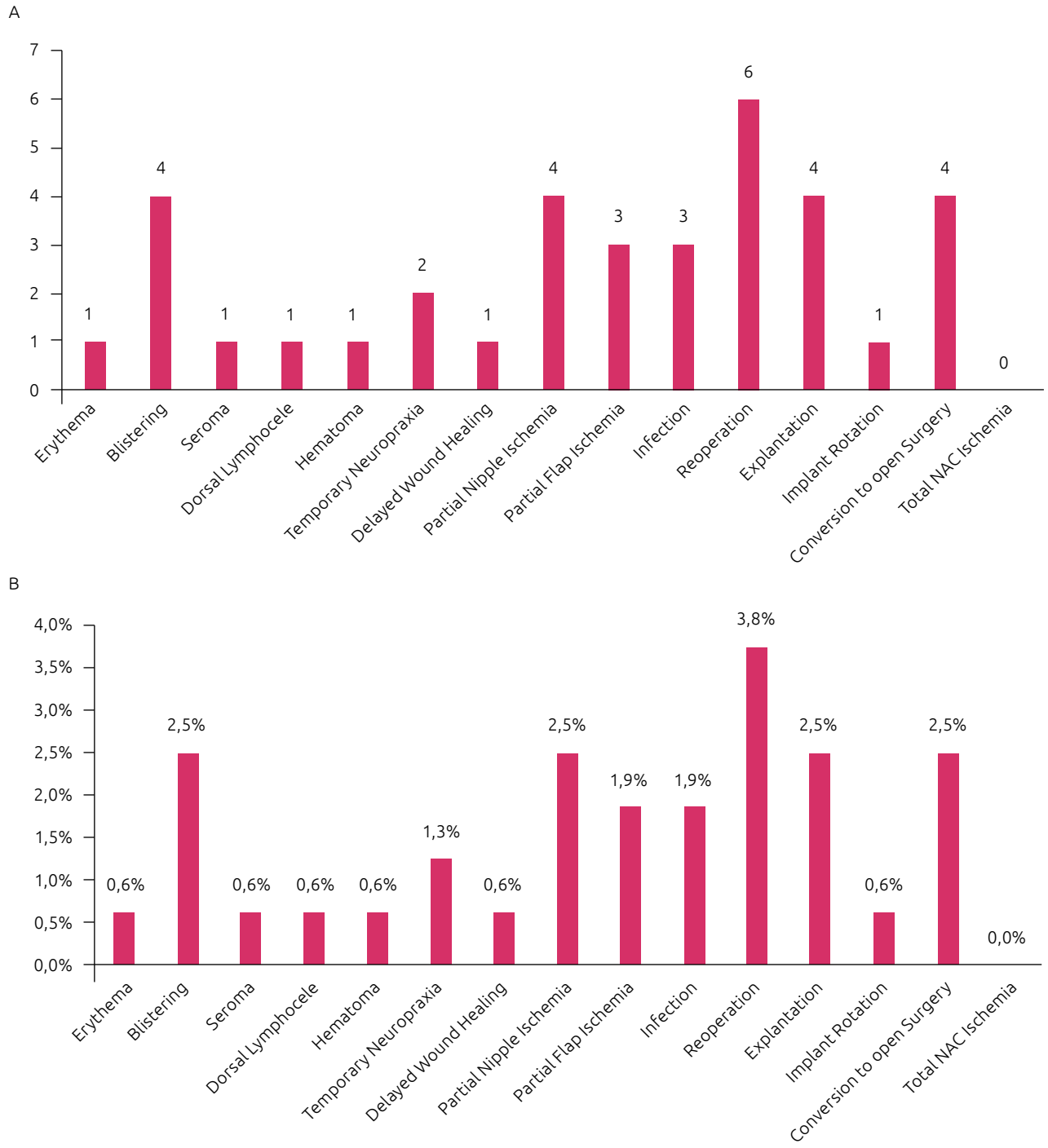

Figure 2. Complications of robotic nipple sparing mastectomy (n = 160): (A) expressed in number of events (total complications = 36; no complications = 124); (B) expressed in percentage (total complications = 22,5%; no complications = 77,5%).

Three-dimensional high resolution optics allow excellent dissection planes. Image magnification and intense lighting increase contrast of colors and visibility of structures, making dissection of the gland and recognition of all structures, especially blood vessels, more precise, thus reducing bleeding and preserving circulation to the nipple areolar complex. High precision movement, stability due to tremor elimination, articulation and motion of instruments enable good mobility around the curvature of the breast cupola[7,9,10].

Complication rates for RNSM are low (23%), mostly minor ones, with only 3% of conversion and 4% of reoperations. Ischemia and necrosis are rare (5%), and no total skin or NAC necrosis were reported.

There are no studies so far that analyze cost-effectiveness for robotic breast surgeries, but the fast learning curve helps to reduce operating room time and consequently the costs. Robotic instruments are known to be expensive, so as maintenance for the robot, but strategies have been proposed to reduce costs[17] and soon new competitors for the Da Vinci are expected to enter the robotic market[20].

In the search for increasingly less invasive surgeries, robotics seems to meet what is proposed without compromising oncological safety and keeping up with high-satisfaction aesthetic results. Longer follow-up and cost-effective analyzes will determine if this technique will be consolidated.

AUTHORS' CONTRIBUTION

P.C. : Conceptualization, Data curation, Formal analysis, Project administration, Writing – original draft.
D.M.P. : Conceptualization; Project administration, Writing – review & editing.
N.C.S.: Conceptualization, Data curation; Writing – review & editing.
J.M.C. : Investigation, Visualization.
F.S.O. : Methodology; Visualization.

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CASE REPORT
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Forequarter amputation in a patient with locally advanced recurrent breast carcinoma

René Aloisio da Costa Vieira[1,2,3], Eduardo Areas Toller[4], Andréa Moreno Morgan[1,5], Idam de Oliveira-Junior[2,5]

ABSTRACT

Forequarter amputation (FQA) involves the removal of the upper limb, clavicle, and scapula and is indicated for the resection of primary or metastatic tumors invading the axillary neurovascular bundle. Reports on breast cancer have associated FQA with the primary resection of a locally advanced tumor, resection of recurrent disease, brachial plexus injury, Stewart-Treves syndrome, or sarcoma secondary to breast cancer irradiation. We described a case of recurrent breast carcinoma with curative-intent surgery. The surgery aimed at locoregional control and improvement in the quality of life. The literature is scarce on the topic, discussing the multiple aspects related to the indication of FQA for breast cancer patients. This report presents the first case described in Latin American literature.

KEYWORDS: Disarticulation; Amputation; Breast neoplasms.

INTRODUCTION

Surgeries that treat tumors of the shoulder girdle are extensive. Forequarter amputation (FQA) involves the removal of the upper limb, clavicle, and scapula and is indicated for the resection of primary or metastatic tumors invading the axillary neurovascular bundle. Although often described in cases of Stewart-Treves syndrome, post-mastectomy sarcomas, and lymphedema, this surgery is rarely reported in carcinomas. Reports on breast cancer have associated FQA with the primary resection of a locally advanced tumor[1], resection of recurrent disease[2-5], brachial plexus injury[5], Stewart-Treves syndrome[6], or sarcoma secondary to breast cancer irradiation[7,8]. The literature is scarce on the topic, and the surgery aimed at locoregional control and improvement in the quality of life, justifying this publication.

CASE REPORT

Female, 73 years old, clinical stage T4bN3M0, associated with extensive and limiting lymphedema of the right upper limb (Figure 1A). Although hypertension was her only comorbidity, the patient was clinically classified as grade 2 in the Eastern Cooperative Oncology Group (ECOG) Performance Status. The biopsy revealed a triple-negative invasive ductal carcinoma of histological grade 3. Initially, the patient underwent two cycles of neoadjuvant chemotherapy with paclitaxel, not responding to therapy and developing febrile neutropenia. Chemotherapy was suspended due to the worsening of her general condition (ECOG grade 3), asthenia, and inappetence. In this context, the treatment chosen was surgery, and the patient was submitted to a right-sided Halsted mastectomy, considered R1 (minimal microscopic disease) because of the disease located along the brachial plexus (Figure 1). Adjuvant radiotherapy was considered for local control, but the presence of surgical wound dehiscence prevented this treatment. Two months later, she showed visible macroscopic recurrence next to the skin of the axillary fossa, leading to the performance of an R1 resection of the region affected by the neoplasm, adjacent to the dehiscence area, with external oblique myocutaneous rotation flap to close the surgical wound

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and provide conditions for adjuvant radiotherapy. She presented new local dehiscence and, in the healing stage, new macroscopic local recurrence (Figures 1 and 2).

Thus, due to the impossibility of administering adjuvant radiotherapy and the early recurrence, FQA was chosen for local control and potential improvement in her quality of life, since the upper limb was no longer functional. FQA was considered R0 (complete resection; Figure 2), and the surgical progress was satisfactory, allowing the start of adjuvant radiotherapy. The patient was questioned about her general quality of life (scores from 1–terrible to 7–great) in the preoperative period, as well as one and three months after surgery. She reported a score of 3 in the preoperative period and 5 in the first and third months. Four months after surgery, she was asymptomatic but showed weight loss of 18 kg, and developed local recurrence metastasis and lung metastasis, being referred to exclusively palliative treatment (Figure 3). Seven months after the FQA, the patient died of pulmonary metastatic disease. FQA has improved her quality of life.

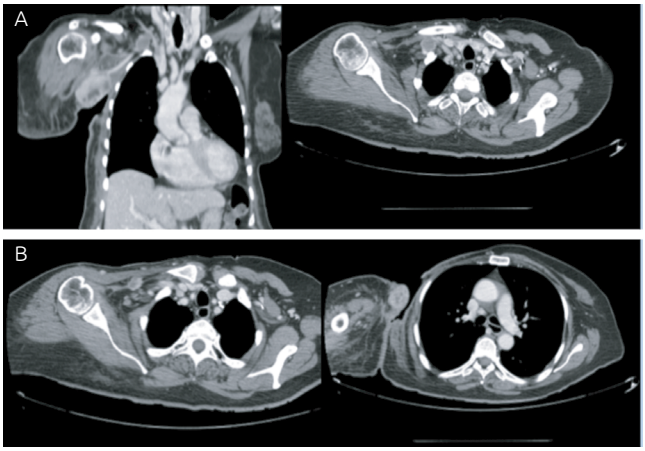

Figure 1. Chest computed tomography (A) pre-treatment; (B) after breast lesion resection with minimal residual extrathoracic disease.

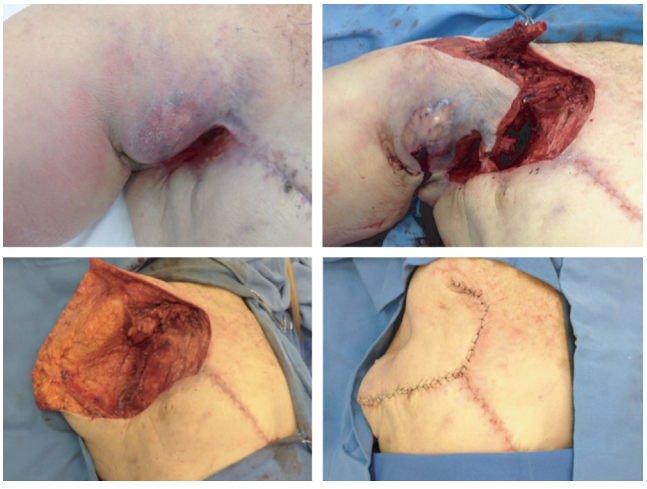

Figure 2. Forequarter amputation.

DISCUSSION

In patients submitted to axillary treatment, recurrence is a rare phenomenon, and, even with surgical treatment, the R1 resection[9] is not often complete. These patients require adjuvant therapies, such as chemotherapy and radiotherapy[9,10], for long-term control of the disease. In some individuals, FQA may be necessary for locoregional control[2,4].

FQA is often performed in cases of tumor of the shoulder girdle[11]. This procedure is usually carried out with curative or palliative intent, allowing locoregional control of the disease and improving the quality of life. Reports on breast cancer have associated FQA with the primary resection of a locally advanced tumor[1], resection of recurrent disease[2-5], brachial plexus injury[5], Stewart-Treves syndrome[6], or sarcoma secondary to breast cancer irradiation[7,8]. In series of this type of surgery, the association with breast cancer represents, on average, 12.5% of the causes[11], an incidence that increases (37.5%) when considering the presence of metastatic disease[12]. Recurrence is its main indication[2-5,12] with palliative intent[3,5]. The literature is scarce on the topic, and we found no cases described in Latin American literature.

Despite the radical nature of the surgery, it allows locoregional control, improvement in symptoms and quality of life, and prolongation of the disease-free interval, which justify its performance in selected cases with curative or palliative intent[2,3,5]. Similarly, this procedure should be considered for patients with brachial plexus injury, neurovascular involvement, and upper limb dysfunction[5].

In the present case, the initial surgery showed the presence of disease along the brachial plexus, and, at first, surgery was not indicated, as radiotherapy was contemplated for local control. Unfortunately, the patient progressed to local dehiscence. Initially, the abdominal oblique flap was considered for primary closure. The new dehiscence, the impossibility of administering other adjuvant therapy, and the local progression of the disease led to the performance of a curative-intent FQA, but the patient

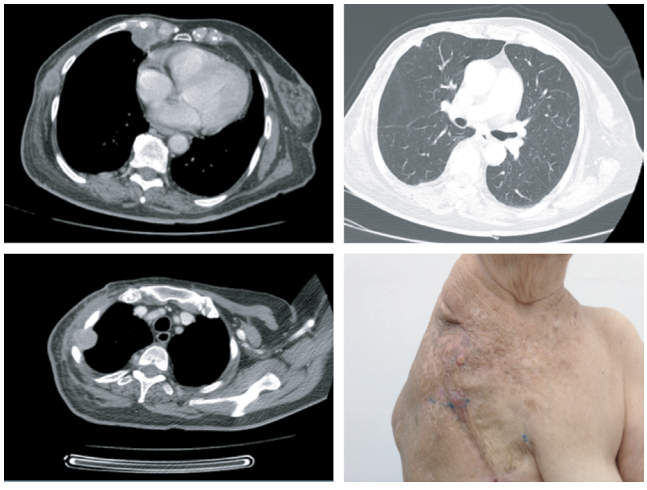

Figure 3. Local and lung recurrence.

died seven months later due to the progression of the lung disease. Usually, FQA is indicated for patients with distant recurrence and prolonged disease-free interval[3]; however, the complications and the clinical condition of the patient led to surgical treatment being the only option for local control.

One of the main points to consider with respect to FQA is the closure of the resected area, which can be done with skin grafts, reuse of part of the skin of the limb, and myocutaneous rotation flaps[2,3,5]. The complication rate is relatively low and usually associated with skin necrosis, local dehiscence, and pleural effusion[2-5]. In this case, the local flaps used originated from the healthy skin of the shoulder, careful of the small area of local dehiscence, controlled with resuture and dressings.

FQA has not been evaluated yet regarding the breast cancer tumor subtype. Triple-negative tumors show worse behavior, but studies involving FQA did not assess this fact. Survival is better in curative-intent treatments, with a mean of 23 months, decreasing to 13 months in palliative ones[3], which fully justifies the surgery in selected cases. In this patient with a triple-negative tumor, FQA was considered curative because of the R0 resection; however, her clinical conditions were poor. The lack of adjuvant therapy and the aggressive nature of the tumor influenced the local recurrence and the short disease-free interval, resulting in limited survival.

CONCLUSION

FQA is an exceptional procedure for patients with recurrent breast carcinoma. It is associated with low surgical morbidity and mortality and should be considered, even if with palliative intent, for prolonging the disease-free interval and improving symptoms of specific diseases and the quality of life.

AUTHORS' CONTRIBUTION

R.A.C.V.: study concept, data curation, formal analysis, methodology, project management.
E.A.T.: data curation, research, methodology.
A.M.M.: methodology.
I.O.-Jr.: formal analysis, methodology.
All authors contributed to the writing of the original manuscript, in addition to reviewing and editing the article.

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