Immunohistochemical profile and clinical-pathological variables in breast cancer

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Summary

Objective: To describe the main characteristics of women with breast cancer, according to the immunohistochemical profile. **Methods:** The population comprised a hospital cohort, consisting of women diagnosed with breast cancer between 2003 and 2005 (n = 601) and treated at a referral center for cancer care in Juiz de Fora, MG, Brazil. Only 397 women who had complete immunohistochemistry analysis were selected. To define the groups according to the immunohistochemical profile, the assessment of estrogen and progesterone receptors, Ki-67 cell proliferation index, and overexpression of human epidermal growth factor receptor 2 (HER2) was chosen. According to the different phenotypes, five subtypes were defined: luminal A, luminal B HER2 negative, luminal B HER2 positive, triple negative, and HER2 overexpression. **Results:** Most patients were white (80.7%) and post-menopausal (64.9%), with a mean age of 57.4 years (± 13.5). At diagnosis, 57.5% had tumor size ≥ 2.0 cm, and 41.7% had lymph node involvement. The most common subtypes were luminal B - HER2 negative (41.8%) and triple negative (24.2%). In the luminal A subtype, 72.1% of patients were post-menopausal, while the highest percentage of premenopausal women were observed in the luminal B - HER2 positive and triple negative subtypes (45.2% and 44.2%, respectively). A higher frequency of tumors > 2.0 cm and lymph node involvement was observed in triple negative and HER2 positive subtypes. **Conclusion:** This study allowed the distribution assessment of the main clinical and pathological characteristics and those related to health services in a cohort of Brazilian women with breast cancer, according to the immunohistochemical tumor subtypes.

Keywords: Progesterone receptors; erbB2 receptor; breast neoplasias; immunohistochemistry; estrogen receptors; Ki-67 antigen.

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Introduction

Breast cancer is the neoplasia with the highest incidence in the female population worldwide[1-3]. Regarding mortality, this neoplasia represented approximately 13.7% of all deaths caused by cancer worldwide in 2008, except for non-melanoma skin tumors[2]. In the U.S. population, there was a 12.3% reduction in mortality rates due to breast cancer between the years 1991 and 2006, which was mainly attributed to the expansion of mammographic screening[4].

In Brazil, approximately 49,240 new cases of breast cancer were estimated in 2010[5]. Based on data from cancer registries of the national population basis, the incidence of the disease is similar to that observed in developed countries[6,7]. It is the leading cause of female death by cancer in the country[8,9], with an estimated 11,735 deaths due to the disease in 2008[5]. According to the Health Surveillance Secretariat, the female population at greatest risk of illness is between the ages of 50 and 69 years. However, there are still significant limitations for these women to have access to secondary preventive measures related to this type of cancer[10].

Tumor heterogeneity of breast cancer is one of the biggest challenges to be faced, considering that tumors with the same histological types, stages, and degrees of differentiation may have different outcomes in relation to prognostic factors and responses to implemented treatments[11,12]. It has been observed that, for better understanding and characterization of breast tumors, the currently used traditional classification has proved to be insufficient[12]. A comprehensive approach is required, including the morphological characteristics; evaluation of tumor aggressiveness, with special reference to histological type; presence of inflammatory response; number of mitoses; nuclear polymorphism; and vascular and lymphatic endothelial involvement[13-15]. It is believed that the differences demonstrated in the biological behavior of microscopically similar tumors can be justified by the complexity of breast cancer and by the accumulation of molecular alterations[15,16].

The advances made in molecular biology techniques have provided better understanding of the mechanisms that regulate cell differentiation and proliferation[17,18]. Accumulation of mutations, genetic alterations, and chromosomal instabilities that stimulate proliferation and cell damage continuously impair the system of growth regulation and apoptosis, and cause the appearance of cancer. These factors have often been recognized and new predictive and prognostic biomarkers have been tested in tumor samples, through immunohistochemical analysis[11,19].

The expression of hormone receptors (estrogen receptors - ER, and progesterone receptors - PR), as well as overexpression or amplification of the human epidermal growth factor receptor 2 (HER2) have been identified as important predictors among patients with breast cancer[20,21]. Currently, these markers are commonly used to define treatment and establish disease prognosis associated with clinical and pathological variables, such as lymph node involvement, tumor size, histological type, tumor grade, and surgical margins[22,23].

Approximately two-thirds of breast tumors express activation for ER and PR in the tumor core and thus are candidates for antiestrogen therapy[24]. Another 20% have HER2 amplification and may benefit from trastuzumab-directed targeted therapy, which is a monoclonal antibody that may be used alone or in combination with chemotherapy, reducing the risk of relapse by 50%, when used as an adjuvant treatment[25-27].

With a better understanding of the structure of the human DNA sequence and the development of high-tech methods such as cDNA microarrays, major changes in cancer-related research have been possible[28]. Several studies are already using this technique for DNA sequencing to better understand the great diversity found among histologically similar tumors[29].

The definition according to the immunohistochemical profile is based on the evaluation of ER and PR, HER2 overexpression, and the Ki-67 cell proliferation index (a monoclonal antibody that detects a nuclear antigen, expressing cells entering the cell cycle and measuring the cell growth fraction)[30-33]. According to the different phenotypes obtained, five subtypes are currently defined: luminal A (ER+, PR+, HER2-), luminal B HER2 negative (ER+ and/or PR+, HER2-), luminal B HER2 positive (ER+ and/or PR+, HER2+), triple negative (ER-, PR-, HER2-) and HER2 (ER-, PR-, HER2+)[30]. Luminal tumors have been associated with a more favorable prognosis, while triple-negative subtypes and HER2 overexpression, with a less favorable prognosis[34,35]. Triple negative tumors have a higher risk of recurrence within three years and higher mortality rates within five years, when compared to other subgroups[36,37].

Considering the current implications of the therapeutic approach for breast cancer, studies that provide a better understanding of the selection of the most appropriate markers to be used in clinical practice in Brazil should be encouraged, as well as a better understanding of the disease distribution in Brazilian women. This study aimed to evaluate the distribution of the main characteristics in women with breast cancer according to the hormone receptor profile (ER and PR), HER2 expression, and Ki-67 proliferation index, by immunohistochemical analysis.

Methods

Study population

The study population consisted of a hospital-based cohort comprising women diagnosed with breast cancer between January 2003 and December 2005 (n = 601) treated at a

referral center for cancer care in the city of Juiz de Fora, state of Minas Gerais, Brazil. The immunohistochemical profile analysis showed that 89 cases had an incomplete profile (absent HER2 and/or Ki-67), and 115 cases did not display such information in the medical files; therefore, only women who had a complete immunohistochemical panel were selected for this study (n = 397).

Data collection and study variables

The recruitment of cases was performed from the cancer registry of the aforementioned hospital-based referral center. Data were collected through an active search of medical records, in order to collect information from previously identified patients using a standardized form.

The immunohistochemical profiling of tumors was performed based on the results of reports issued by pathology services with renowned technical quality, based on the evaluation of ER and PR, HER2 overexpression, and Ki-67 proliferation index. According to the different phenotypes obtained, five immunohistochemical subtypes were defined: luminal A (ER+, PR+, HER2-), luminal B HER2 negative (ER+ and/or PR+, HER2-), luminal B HER2-positive (ER+ and/or PR+, HER2+), triple negative or basal (ER-, PR-, HER2-), and HER2 overexpression (ER-, PR-, HER2+)[30]. According to the 2011 St. Gallen Consensus, the Ki-67 index is considered low or negative when < 14%, and positive or high when ≥ 14%[29]. In the study population, however, this marker was scored as null (no immunostaining), low (10% or less immunopositivity), or high (> 10% immunoreactive cells), based on the criteria adopted at the time of diagnosis of the cases[38]. For this study, the Ki-67 index was considered low for cases with an immunopositivity value < 10%, and high for those with values ≥ 10%.

The following variables were analyzed: date of diagnosis; age at diagnosis (in years) categorized as: up to 39, 40-49, 50-69, and ≥ 70 years, dichotomized as ≤ 50, and > 50 (cutoff validated as a marker for menopausal status)[39]; ethnicity (classified as white or non-white). Variables related to healthcare services were: the nature of the oncology service (public, or private); presence of private health insurance; type of surgery (curative or diagnostic only, excisional biopsy, or lumpectomy without lymph node approach); additional therapy (radiotherapy, chemotherapy, or hormone therapy). Tumor characteristics were: tumor size (categorized as: ≤ 2.0 cm and > 2.0 cm); histological type; lymph node involvement; staging according to TNM classification of the Union for International Cancer Control (UICC)[40]; and presence of metastases (locoregional or systemic; at diagnosis and during the course of the disease - the latter obtained by information collected at the time of data collection carried out in 2010).

Data analysis

EPI INFO, release 3.5.3 (2011), was used for data entry and analysis. The differences in the distribution of the study variables were evaluated by the chi-square test (χ^2) and, when necessary, by Fisher's exact test (SPSS 8.0), considering statistically significant when p-value < 0.05. The study design was approved by the Ethics Committee of Universidade Federal de Juiz de Fora - protocol No. 042/2008.

Results

According to the immunohistochemical profile, the study population was distributed in the following subtypes: luminal A: 17.1%; luminal B, HER2 negative: 41.8%; luminal B, HER2 positive: 10.8%; HER2 overexpression : 6.0%; and triple negative: 24.2%. The predominant histological type was infiltrating ductal (73.3%), followed by infiltrating lobular (9.8%), and other histological variants (8.6%), with 7.0% of the cases represented by *in situ* carcinoma.

The distribution of the main clinical characteristics according to breast cancer subtypes classified by immunohistochemistry is shown in Table 1. The mean age at diagnosis was 57.4 years (range 26-91 years), with a median of 58.0 years (25th percentile: 46.0, and 75th percentile: 67.0), and 73% of these were between 40 and 69 years. Only 27 women (6.9%) were younger than 39 years. Among patients with HER2 overexpression, two peaks of higher frequency of cases were identified: between 40 and 59, and 70 years or more.

Most women were white (80.7%), with a higher percentage of non-white in the triple negative subtype (39.7%), and white in the luminal B HER2 negative subtype (45.4%) (p = 0.02).

The age dichotomized for the characterization of menopausal status showed that 64.9% of patients were postmenopausal. For the luminal A subtype, it was observed that 72.1% of patients were postmenopausal, whereas luminal B HER2 positive and triple negative subtypes showed the highest percentages of premenopausal women (45.2% and 44.2% respectively, p = 0.07).

The pathological features according to the subtypes considered are shown in Table 2. At diagnosis, 57.5% of patients had tumors > 2.0 cm, and 41.7% had lymph node involvement. The highest percentages of tumors > 2.0 cm were observed in HER2 overexpression, luminal B HER positive, and triple negative subtypes (78.9%, 70.0% and 60.6%, respectively), whereas among the luminal A subtype, tumor size ≤ 2.0 cm was observed in 58.7% of cases (p = 0.009). It is noteworthy that the highest percentages of tumors > 5 cm were found in luminal B HER2 positive and triple negative subtypes (17.5% and 13.8% respectively, p = 0.03 - data not shown).

Among the subgroups involved with higher frequency of lymph node involvement, luminal B HER2 positive (53.8%), triple negative (48.9%), and HER2 overexpression

Table 1 – Distribution of clinical characteristics according to subtypes of breast cancer classified by immunohistochemical analysis

Characteristics	Luminal A	Luminal B-HER2 negative	Luminal B-HER2 positive	HER2	Triple negative	Total cases and %	p-value
Race*							0.02
White	50	129	28	17	60	284	
Line %	17.6	45.4	9.9	6.0	21.1	100	
Col %	89.3	84.9	77.8	81.0	69.0	80.7	
Non-white	6	23	8	4	27	68	
Line %	8.8	33.8	11.8	5.9	39.7	100	
Col %	10.7	15.1	22.2	19.0	31.0	19.3	
Age at diagnosis#							0.5
< 40	2	14	6	1	4	27	
Line %	7.4	51.9	22.2	3.7	14.8	100	
Col %	2.9	8.5	14.3	4.2	4.2	6.9	
40-49	16	36	11	6	34	103	
Line %	15.5	35.0	10.7	5.8	33.0	100	
Col %	23.5	22.0	26.2	25.0	35.8	26.2	
50-59	15	38	7	6	19	85	
Line %	17.6	44.7	8.2	7.1	22.4	100	
Col %	22.1	23.2	16.7	25.0	20.0	21.6	
60-69	21	44	8	5	21	99	
Line %	21.2	44.4	8.1	5.1	21.2	100	
Col %	30.9	26.8	19.0	20.8	22.1	25.2	
≥ 70	14	32	10	6	17	79	
Line %	17.7	40.5	12.7	7.6	21.5	100	
Col %	20.6	19.5	23.8	25.0	17.9	20.1	
Menopausal status*							0.07
Post-menopausal	49	113	23	17	53	255	
Line %	19.2	44.3	9.0	6.7	20.8	100	
Col %	72.1	68.9	54.8	70.8	55.8	64.9	
Pre-menopausal	19	51	19	7	42	138	
Line %	13.8	37.0	13.8	5.1	30.4	100	
Col %	27.9	31.1	45.2	29.2	44.2	35.1	

*χ^2 Test; #Fisher's Test. Total cases of each variable may differ due to the occurrence of ignored data.

(40.9%) were noteworthy, whereas luminal A showed the lowest percentage (12.3%) (p = 0.05). A higher frequency of tumors > 2.0 cm and lymph node involvement was observed in triple negative, luminal B HER2 positive, and HER2 overexpression subtypes.

For the luminal A subtype, 92% of tumors showed early stage disease (*in situ*: 7.9%, I: 44.4%, II: 39.7%), while for the other subgroups, there were higher percentages in stages II and III (luminal B HER2 positive: 72.1%; triple negative: 71.2%, HER2 overexpression: 63.6%, luminal B HER2 negative: 61.5%). At diagnosis, an increased frequency of disease in stage IV was identified for the HER2 overexpression subtype (9.1%). Regarding menopausal status, there were higher percentages of cases at early stages in postmenopausal women (*in situ*: 73.9%, I: 72.1%, II: 63.6%), while premenopausal women exhibited higher rates of advanced disease (III and IV: 48.8% and 21.4%, respectively, p = 0.02 - data not shown).

Metastatic disease at diagnosis was demonstrated in 15 women (4.6%), some with more than one metastatic site involved, and the most common sites were: bone (70.5%), liver (23.5%), and lung (17.6%). During the course of the disease, 15.9% of patients (n = 63) developed systemic dissemination. Among the subtypes more

Table 2 – Distribution of pathological characteristics according to subtypes of breast cancer classified by immunohistochemical analysis

Characteristics	Luminal A	Luminal B-HER2 negative	Luminal B-HER2 positive	HER2	Triple negative	Total cases and %	p-value
Tumor size#							0.009
≤ 2.0 cm	37	68	12	4	37	158	
Line %	23.4	43.0	7.6	2.5	23.4	100	
Col %	58.7	43.6	30.0	21.1	39.4	42.5	
> 2.0 cm	26	88	28	15	57	214	
Line %	12.1	41.1	13.1	7.0	26.6	100	
Col %	41.3	56.4	70.0	78.9	60.6	57.5	
Lymph nodes*							0.05
Negative	48	90	18	13	46	215	
Line %	22.3	41.9	8.4	6.0	21.4	100	
Col %	71.6	59.6	46.2	59.1	51.1	58.3	
Positive	19	61	21	9	44	154	
Line %	12.3	39.6	13.6	5.8	28.6	100	
Col %	28.4	40.4	53.8	40.9	48.9	41.7	
Metastases (at diagnosis and during the course of the disease)*							0.0005
Absent	62	139	38	14	69	322	
Line %	19.3	43.2	11.8	4.3	21.4	100	
Col %	91.2	83.7	88.4	58.3	71.9	81.1	
Present	6	27	5	10	27	75	
Line %	8.0	36.0	6.7	13.3	36.0	100	
Col %	8.8	16.3	11.6	41.7	28.1	18.9	
Staging#							0.001
In situ	5	11	3	3	1	23	
Line %	21.7	47.8	13.0	13.0	4.3	100	
Col %	7.9	7.1	7.0	13.6	1.1	6.1	
I	28	43	8	3	23	105	
Line %	26.7	41.0	7.6	2.9	21.9	100	
Col %	44.4	27.6	18.6	13.6	24.5	27.8	
II	25	67	17	11	35	155	
Line %	16.1	43.2	11.0	7.1	22.6	100	
Col %	39.7	42.9	39.5	50.0	37.2	41.0	
III	3	29	14	3	32	81	
Line %	3.7	35.8	17.3	3.7	39.5	100	
Col %	4.8	18.6	32.6	13.6	34.0	21.4	
IV	2	6	1	2	3	14	
Line %	14.3	42.9	7.1	14.3	21.4	100	
Col %	3.2	3.8	2.3	9.1	3.2	3.7	

#Fisher's Test; *χ^2 Test. Total cases of each variable may differ due to the occurrence of ignored data.

involved with increased morbidity, the most important were: HER2 overexpression (50.0%), and triple negative (48.0%), compared to luminal A (13.8%) and luminal B HER2 positive (19.2%) subtypes, and the latter two were related to lower incidence of distant metastases (p = 0.004 - data not shown).

Among the 16 (7.3%) women who had local and regional disease recurrence, the HER2 positive and triple negative subtypes were also responsible for the highest percentage (14.3% and 12.2%, respectively), whereas in the luminal A subtype only one patient was identified with local recurrence. There was no significant difference in the distribution of metastases (at diagnosis and during the course of disease) in the study population, according to the nature of the health service (public *versus* private - data not shown).

The distribution of characteristics related to the use of health services is shown in Table 3. The highest percentage of cancer patients used the private healthcare system (56.4%). Among the HER2 overexpression, luminal B HER2 negative, and luminal A subtypes, most patients were treated by private healthcare services (83.3%, 59.0% and 54.4%, respectively), whereas patients with luminal B HER2 positive and triple negative subtypes showed to be evenly distributed between the public health system (Sistema Único de Saúde – SUS) and private healthcare services (p = 0.03). Among the women treated by the public health service, 5.6% had healthcare insurance.

Most women underwent surgery with curative intent (93.8%). A higher percentage of curative surgery among patients with stage I (99.0%), II (92.9%) and III (97.4%), and diagnostic surgery among those with stage IV (53.8 %) (p = 0.000) was observed, with no statistically significant difference in the distribution of cases regarding the indication for surgery (curative or diagnostic), according to established immunohistochemical subtypes. With regard to systemic treatment (chemotherapy and /or hormone therapy), 95.1% of the patients received some type of therapeutic approach, with 60.6% receiving chemotherapy (n = 238), and 60.3% hormone therapy (n = 237).

Extensive use of hormone therapy was observed among patients with luminal subtypes (A: 88.2%; B HER2 negative: 84.8%; and B HER2 positive: 76.7%), whereas this use was minimal among HER2 overexpression and triple negative subtypes (0.4% and 1.7%, respectively) (p = 0.000). Among the subtypes, there was a higher percentage of chemotherapy use with triple negative (78.7%) and HER2 overexpression (70.8%), whereas among cases of luminal A subtype, only 36.8% of cases used this therapy (p = 0.000).

Among the cases of luminal B HER2 positive subgroup, 34.9% of patients did not use chemotherapy. Radiotherapy was used in 80.9% of patients, especially among those who had HER2 overexpression subtypes (95.0%) and luminal B HER2 positive (90.0%), who were in the subgroups with more advanced disease at diagnosis (tumors > 2.0 cm and lymph node involvement), compared with those who did not receive this therapy (p = 0.01).

Discussion and conclusion

This study identified the distribution of immunohistochemical subtypes in patients with breast cancer who underwent treatment in public and private healthcare institutions of a medium-sized city, a macro-regional reference center for cancer care in Southeastern Brazil. According to this profile, the immunohistochemical subtypes with higher frequencies were luminal B HER2 negative (41.8%) and triple negative (24.2%). This finding differs from that observed in a study of 10,159 women, based on data from 12 cancer registries and a hospital-based population of several countries (North America, Europe, and Australia), with a case reporting period from 1974 to 2005, which demonstrated that luminal A was the most frequent subtype, with a percentage of approximately 71.3%, followed by triple negative (16%)[41].

The latter study did not include the evaluation of the Ki-67 cell proliferation index, and instead considered other markers for classification of subtypes, such as epidermal growth factor receptor (EGFR) and cytokeratin 5 and/or 6. In the present study, however, the classification proposed by the St. Gallen Consensus (2011) was considered, which stratified the tumors as luminal A, B HER2 negative, and B HER2 positive, taking into account a cutoff of 14 % for the Ki-67 cell proliferation index. However, in the present study, the Ki-67 index was considered low for cases with value < 10% of immunopositivity and high for those with value ≥ 10%, as, in this population, this marker was scored as null (no immunostaining), low (≤ 10% of immunopositivity), or high (> 10% immunoreactive cells), based on the criteria adopted at the time of diagnosis[38]. This fact may explain the high percentage of tumors classified as luminal B HER2 negative in this study.

The triple negative subtype, which in most studies shows frequency between 10% and 20%[31,35,41,42], was found in almost one-quarter of the population (24.2%). This subtype showed higher percentages in younger patients, aged < 50 years (40%) and non-white (39.7%). These findings are similar to those observed in other studies that have persistently shown the prevalence of triple negative tumors in younger black patients displaying more advanced disease at diagnosis and higher cell proliferation index[35,43]. Together with the triple negative subtype, the HER2 overexpression and luminal B-HER2 positive subgroups also showed, in this study, higher percentages of non-white patients with more advanced initial disease. Noteworthy is the fact that the subtypes that express HER2 are strongly influenced by the proliferative state of the tumor, which tends to be higher in those subgroups[44], which may explain their association with tumors presenting more aggressive behavior.

The predominant histological type was infiltrating ductal (73.5%), similar to the findings of other studies performed to assess survival in women with breast cancer

Table 3 – Distribution of characteristics related to use of the public health services and treatment, according to subtypes of breast cancer classified by immunohistochemical analysis

Characteristics	Luminal A	Luminal B HER2 negative	Luminal B HER2 positive	HER2	Triple negative	Total cases and %	p-value
Health service#							0.03
Private	37	98	21	20	48	224	
Line %	16.5	43.8	9.4	8.9	21.4	100	
Col %	54.4	59.0	48.8	83.3	50.0	56.4	
Public	31	68	22	4	48	173	
Line %	17.9	39.3	12.7	2.3	27.7	100	
Col %	54.6	41.0	51.2	16.7	50.0	43.6	
Surgery#							0.2
Curative	53	126	37	16	82	314	
Line %	16.9	40.1	11.8	5.1	26.1	100	
Col %	80.3	79.2	90.2	72.7	86.3	82.0	
Diagnostic	13	33	4	6	13	69	
Line %	18.8	47.8	5.8	8.7	18.8	100	
Col %	19.7	20.8	9.8	23.7	13.7	18.0	
Hormone therapy#							0.0000
Use	60	139	33	1	4	237	
Line %	25.3	58.6	13.9	0.4	1.7	100	
Col %	88.2	84.8	76.7	4.2	4.3	60.3	
Non-use	8	25	10	23	90	156	
Line %	5.1	16.0	6.4	14.7	57.7	100	
Col %	11.8	15.2	23.3	95.8	95.7	39.7	
Chemotherapy*							0.0000
Use	25	94	28	17	74	238	
Line %	10.5	39.5	11.8	7.1	31.1	100	
Col %	36.8	57.3	65.1	70.8	78.7	60.6	
Non-use	43	70	15	7	20	155	
Line %	27.7	45.2	9.7	4.5	12.9	100	
Col %	63.2	42.7	34.9	29.2	21.3	39.4	
Radiotherapy#							0.01
Use	57	116	36	19	69	297	
Line %	19.2	39.1	12.1	6.4	23.2	100	
Col %	86.4	73.4	90.0	95.0	83.1	80.9	
Non-use	9	42	4	1	14	70	
Line %	12.9	60.0	5.7	1.4	20.0	100	
Col %	13.6	26.6	10.0	5.0	16.1	19.1	

#Fisher's Test; *χ^2 Test. Total cases of each variable may differ due to the occurrence of ignored data.

in Brazil[45-48]. The mean age at diagnosis was 57.4 years, a finding similar to that observed in a study by Blows et al.[41], which observed a higher frequency of the disease among women aged 50 to 59 years for all subtypes, although slightly higher than the mean ages found in other Brazilian studies[47,48], who were respectively 54.0 and 56.4 years. Kwan et al.[49] found a higher frequency of younger women with the triple negative subtype, a finding similar to the present study, which identified a percentage of 35.8% of this subtype in those aged between 40 and 49 years.

Most women were white (80.7%), with a higher frequency among all subtypes. The black race is, in general, associated with worse prognosis when compared to other ethnic-racial groups[50]. A higher percentage of non-white with the triple negative subtype (39.7%) was found, which was related with more advanced initial disease and may reflect, for these women, late diagnosis and difficult access to healthcare services. This finding may also represent a greater severity of these tumors due to their phenotype. However, the miscegenation of the Brazilian population should be taken into account (when compared to the U.S. population, for example), which complicates the precise characterization of this variable and, also, the possibility of misclassification of ethnicity in this study, as this information was collected based on the perceptions of the professionals responsible for filling out the initial medical file.

It should be noted that in this population, which had all the markers selected for the study, 56.4% of women were treated by the private health sector. Therefore, it is a distinct population, regarding access to healthcare, in relation to the Brazilian population. For HER2 overexpression, luminal B HER2 negative, and luminal A subtypes, most patients were treated at private healthcare services (83.3%, 59.0%, and 54.4%, respectively). Also noteworthy is the fact that among the patients who were treated at public health services, 5.6% had private healthcare insurance.

A higher frequency of tumors < 2.0 cm (65.8%) and with no lymph node involvement (62.8%) was observed in patients treated at the private healthcare services, while the highest percentages of tumors > 2.0 cm and positive lymph nodes were identified in the public healthcare services (66.3% and 50.6% respectively, $p = 0.003$ and $p = 0.002$). In a survival study carried out by Guerra et al.[51] in a hospital cohort of women with breast cancer treated in the same city, there was a higher frequency of patients who had private healthcare insurance and had been treated at the public healthcare services (37.7%). However, the latter study showed an increased risk of death due to breast cancer in women with no private healthcare, which was related to later diagnosis and poor access to specific treatment, in accordance with what was observed in the present cohort regarding the type of the health services used.

Developing countries present lower incidence of overall cancer rates, (considering all locations), approximately half of that observed in developed countries. However, the mortality rates tend to be similar in these countries[3], which can be a result of a higher death risk in developing countries, probably due to a combination of risk factors in these countries, such as diagnosis at a later stage of the disease, and limited access to diagnostic methods and standard treatment[3,10]. Although the socioeconomic status of the study population can be considered high in relation to the Brazilian population in general, tumors > 2.0 cm were predominant, with positive axillary nodes, reinforcing the need for public health policies aimed at consolidating the national screening program for breast cancer, especially for the group of women considered at higher risk, as well as ensuring timely treatment for diagnosed patients.

Noteworthy is the fact that the high percentage of the luminal B HER2 negative subtype in this cohort may be related to the probable inclusion of an unknown percentage of tumors that should be classified as luminal A, according to the classification prior to that of the St. Gallen Consensus (2011). Thus, using the classification proposed by the Consensus in this study may have favored the combination, in the luminal B HER2 negative category, of the luminal A and B subtypes, with the latter being a subtype often associated with a more favorable outcome. This possibility should be taken into account when interpreting the differences observed in relation to the luminal B HER2 negative subtype.

When interpreting the findings regarding the distribution of metastases in the study population, however, the possibility of metastasis underestimation at diagnosis must be taken into account, as staging tests could not be verified for all cases. In this sense, it is also worth mentioning that there was no significant difference in the distribution of metastases (at diagnosis and during the course of the disease) in the study population, according to the nature of the health service (public *versus* private).

Finally, it must be considered that in this study, immunohistochemical data were obtained from medical reports contained in patient files and examination reviews were not carried out, which can have an impact on the reliability of this information. However, it is worth mentioning that all pathological anatomy services responsible for the immunohistochemical panel assessment of the study population had acknowledged technical quality, were accredited and provided concomitant services to public and private institutions. These findings reinforce data quality and minimize a possible differential error related to the nature of health services.

This study allowed the characterization of immunohistochemical subgroups in patients with breast cancer treated at a referral center for cancer care in Southeastern Brazil, using a recently updated immunohistochemical classification[30]. It also allowed the assessment of subgroup distribution in relation to the main clinical and pathological characteristics and those related to the use of healthcare services. In this sense, it is noteworthy that the association between the histological diagnosis and immunohistochemical technique can help to determine the phenotypic profile of breast cancer, aiming to guide treatment and, consequently, to improve the therapeutic response.

Therefore, the need for better use of available information on health services responsible for the care of cancer patients in Brazil becomes clear, aiming to produce knowledge that can best assist the effort to manage this public health problem in Brazil.

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