Immunohistochemical Profile and Clinical-Pathological Variants of Breast Cancer in Northeastern Mexico

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Abstract: *Background*: Breast cancer is a heterogeneous illness, with subtypes of varying etiology. Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2/neu (Human Epidermal Growth Factor Receptor 2) expressions have been identified as predicting factors.

Objective: To demonstrate the possible association of the five immunohistochemical (IHC) expression profiles with clinical and histopathological variables of breast cancer in northeastern Mexico.

Methodology: In 522 women with breast carcinoma, five IHC profiles were defined [Luminal A, Luminal B, Mixed, HER2/neu and Triple-negative (TN)]. An analysis was done to determine if there were differences between them in relation to the clinical and histopathological variables.

Results: The distribution of the histological subtypes was: luminal A (32.97%), TN (27.53%), HER2/neu (19.02%), mixed (13.41%) and luminal B (7.07%). The average age at diagnosis was 53.07 ± 12.08 years, in 90.5% of the patients the size of the tumor was ≥ 2.0 cm, and 40.94% had lymph node involvement. Luminal A subtype had the highest percentage in the postmenopausal state (63.7%, p=0.071). Illness recurred in 21.01% of the patients (n=116), principally with the TN subtype (28.3%, p=0.012).

Conclusions: This study detected the characterization of IHC subgroups in patients treated for breast cancer at a reference center for cancer treatment in northeastern Mexico.

Keywords: Breast cancer, HER2/neu, estrogen receptor, progesterone receptor, immunohistochemistry.

INTRODUCTION

Breast cancer is the most frequent malignant tumor and the principal cause of death by carcinoma in women, with a large heterogeneity in its clinical presentation [1]. World-wide, breast cancer is by far the most frequent cancer among women, with an estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers); this neoplasm was the 5th cause of death (6.1% of deaths) in 2008 [2]. In Mexico, breast cancer causes a heavy burden due to premature death; 60% of the women who die are 30 to 59 years old. There is also certain evidence that suggests, on average, the illness starts at an earlier age in developing countries than in more developed countries [3, 4]. These phenomena are observed more frequently in northern Mexico, where the epidemiological transition, associated with a combination of biologic, genetic, environmental and lifestyle differences, due to the cultural influence of our neighboring country the United States, could be affecting the epidemiological expression of this illness [Li CI, 2002].

Today it is well known that the clinical and prognostic diversity of breast carcinoma, similar and homogeneous in their classical prognostic factors, is established at a molecular level, where the expression of distinct genes confers this biologic and prognostic variability [5]. Understanding the biological heterogeneity of breast cancer is one of the greatest challenges, as tumors with the same histological type, stages and grades of differentiation may have different results in relation to prognostic factors and response to applied treatments [6, 7]. The classification currently in use seems to be insufficient and unreliable for an adequate understanding and characterization of breast cancer [8]. An integral focus on the tumor is needed, including morphological characteristics, histological type, inflammatory response, immunohistochemical characteristics, number of mitosis, nuclear polymorphism, and vascular and lymphatic involvement [9, 10]. Also, various studies suggest that molecular and racial differences could be associated to the biologic behavior of breast cancer [11].

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Current advances in molecular biology and histopathologic diagnostic techniques have allowed for a greater understanding of the mechanisms that regulate differentiation, cellular proliferation [12], spreading mechanisms [13], signaling pathways [14] and tumor classification [15], permitting treatments to be aimed at specific targets in the neoplastic cells.

The expression of hormonal (estrogen, progesterone) receptors [16] along with the overexpression of the Human Epidermal Growth Factor Receptor 2 (HER2/neu), have been identified as predictive factors in breast cancer [17, 18].

More than half of breast cancer tumors express activity for estrogen receptors (ER) and progesterone receptors (PR) in the tumoral nucleus, and therefore are candidates for hormonal therapy [19]. Another 15-20% amplify for the HER2/neu protein. In this case, adjuvant therapy with Trastuzumab® can be beneficial, a monoclonal antibody given alone or in combination with chemotherapy, reducing the risk of recurrence up to 50% [20].

The definition of the breast cancer intrinsic subtypes is based on the receptor assessment, that is, of ER, PR and HER2/neu [21, 22]. The different combinations of positive and negative results defines the following phenotypes; luminal A (RE+,RP+, HER2/neu-), luminal B (RE+, RP-, HER2/neu-), mixed (RE+, RP+, HER2/neu+), triple negative (RE-, RP-, HER2/neu-), and HER2/neu (RE-, RP-, HER2/neu+) [23, 24]. Luminal A or B tumors have been associated with a more favorable prognosis [25], and the TN and HER2/neu overexpression subtypes with a less favorable prognosis [17, 26]. Also, the presence of the triple-negative subtype, which occurs in less than 20% of the tumors, has an elevated recurrence risk during the first three years after diagnosis, and high mortality rates during the first 5 years of the illness when compared to other groups [27].

The aim of this study was to assess the distribution of the principal characteristics of women with primary breast cancer according to the receptor hormonal profile (ER, PR) and the HER2/neu expression, using IHC analysis.

Taking all this into account, these type studies will permit a better understanding of breast cancer. They will also determine the distribution of the specific subtypes of this illness in the Mexican population, and help improve the choice of the therapeutic protocols.

MATERIALS AND METHODS

Patients and Samples

From 2006 to 2011, a total population of 768 women, diagnosed with primary invasive ductal breast cancer, was detected and referred to the Centro Médico Nacional del Noroeste, in the city of Obregón, Sonora, Mexico. Other subtypes (invasive lobular carcinoma, in situ ductal carcinoma, or special histological types) were excluded. The IHC profile was analyzed, when available, in the selected cases. The final study population consisted of 552 women; 216 cases were eliminated due to an incomplete IHC profile.

Immunohistochemistry and its Assessment

All samples were subjected to IHC tests for ER, PR and HER/2neu. The IHC analysis was done on tumoral tissue fixated with buffered 10% formaldehyde and embedded in paraffin wax. Tissues previously proven to be positive for the markers were used as controls.

The IHC was done with anti-ER (Novocastra, clona: ER-6F11, 1:20 dilution, Newcastle, United Kingdom) and anti-RP (Dako, clona: PgR-636, 1:50 dilution, Glostrup, Denmark). In the specific case of HER2/neu, the evaluation was semi-quantitative; the category scale was 0 to +++, with +++ considered positive, and 0 or + negative. In the cases with ++, another evaluation was done with the FISH technique (fluorescence *in situ* hybridization), using 2 DNA fluorescent probes to determine if it was positive or negative. The final result was obtained calculating the quotient between the number of signals from both probes in a minimum of 20 tumoral cell nuclei. It was considered positive when the amplification quotient had a value higher than 1.8 for HER2/neu.

Definition of Intrinsic Subtypes

The tumoral intrinsic subtypes were classified as luminal A, luminal B, HER2/neu or TN, according to the IHC results for ER, PR and HER2/neu, as defined in the introduction [28].

Histological Grading

Histological grading was obtained using the Scarff-Bloom-Richardson (SBR) system [29]. The tumors were graded independently by two expert pathologists, with a diagnostic inter-observer agreement of over 90% during the first essay. The SBR grading system is

based on the microscopic assessment of morphological and cytological patterns of the tumor cells, including tubule formation, nuclear pleomorphism and mitosis rate. The final sum of these parameters is then used to grade the tumor: grade I (3-5 points, well differentiated), grade II (6-7 points, moderately differentiated) and grade III (8-9 points, poorly differentiated). In all cases, with the exception of HER2/neu, a stain was considered positive when the expression was present in more than 10% of the cells in 10 fields at high resolution.

Clinical and Epidemiological Information

In all cases the clinical variables related to the diagnosis (age, menopause, affected gland) were registered, along with tumor size, TNM staging [30], lymphatic gland involvement, metastasis at the moment of diagnosis or during the course of the illness, type of surgery (conservative or radical), additional treatments (hormonal, adjuvant, radiotherapy or chemotherapy), illness activity, recurrence or persistence, and death.

Statistical Analysis

The data was captured and analyzed with SPSS® version 20.0 for Windows® 2011 (IBM Corporation, Armonk, New York, USA). The intra- and intergroup differences for the dichotomical variables and the percentages were analyzed with Pearson's chi-squared test ($\chi 2$), and when necessary, with Fisher's exact test. Student's *t*-test was used for numeric variables, the ANOVA test for analysis between groups. When the *p* value was equal or less than 0.05 it was considered statistically significant.

Declaration of Ethics

This study was approved by the Local Health Research Committee 2601, of the Hospital de Especialidades No 2, Unidad Médica de Alta Especialidad del Instituto Mexicano del Seguro Social, part of the Centro Médico Nacional del Noroeste, with registry number R-2007-2601-43. All participating subjects signed an informed consent form.

RESULTS

The average age at the moment of diagnosis was 52.72 ± 12.64 years (range 22 - 89 years). Only 81 women (13.60%) were less than 39 years old. The IHC subtype distribution observed in the study population was as follows: luminal A (32.97%); luminal B (7.07%); mixed (13.41%); HER2/neu (19.02%) and TN (27.53%). The average age of women with luminal A subtype was higher (55.0 ± 11.9 years; p = 0.04). The majority of the subjects with subtypes luminal A, mixed, HER2/neu and TN were 40-69 years old. However, in the case of subtype luminal B, we observed two age-related frequency peaks: under 40 years (20.5%) and 50-59 years (30.8%).

When menopause was used as the dichotomizing characteristic, 315 (52.90%) met this criterium. Luminal A and luminal B subtypes had the highest percentages in the post-menopausal state (63.7% and 61.5% respectively). On the other hand, higher percentages of mixed and TN subtypes were observed in the pre-menopausal state (51.4% and 47.4% respectively). The distribution of the clinical characteristics, according to the breast cancer subtypes classified with IHC, are described in Table **1** and Figure **1**.

At the time of diagnosis, 90.5% of the patients had a tumor size ≥ 2.0 cm, and 40.94% had lymph node involvement. A greater proportion of tumors ≥ 2.0 cm were found in subtypes luminal A, TN and HER2/neu (90.1%, 90.1% and 90.5%, respectively; $p = 0.092$). As to lymph node involvement, TN, mixed and HER2/neu subtypes showed the highest percentages of positivity (47.3%, 46.0% and 38%, respectively).

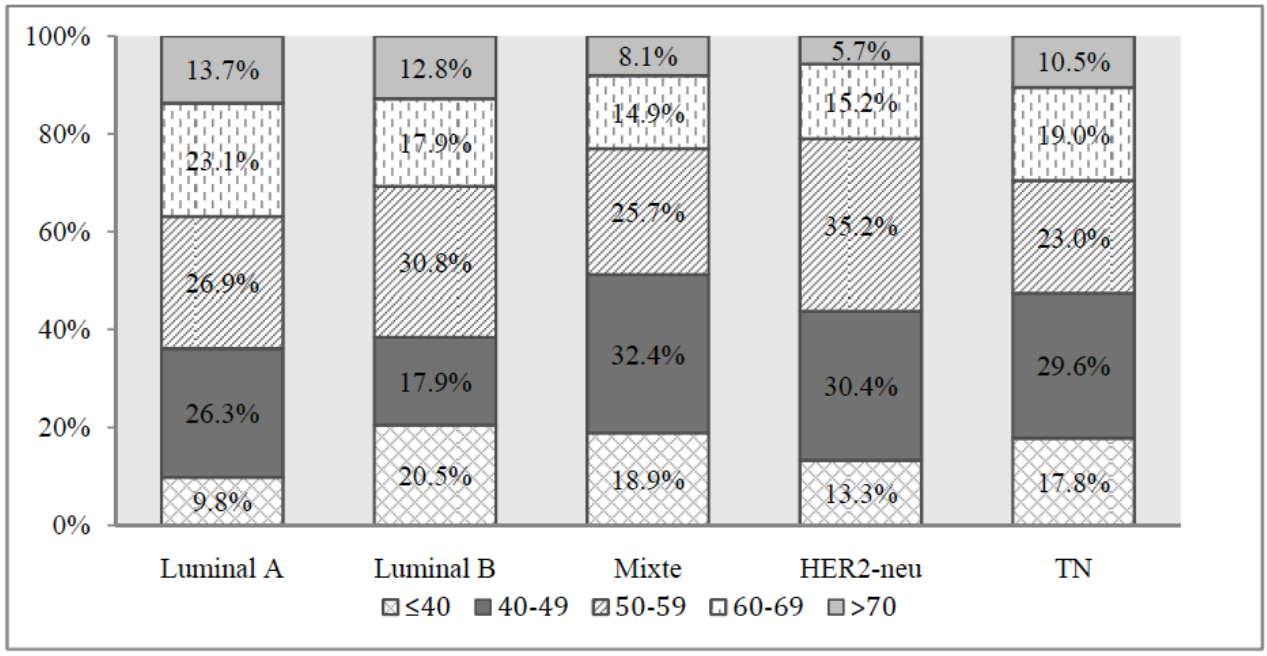
In the mixed subtype, 73.1% of the tumors were in an early stage of the illness (in situ: 12.2%, $p = 0.334$; I: 9.5%, $p = 0.020$; II: 51.4%, $p = 0.043$). In stage IV tumors, high frequencies of subtypes luminal A, TN and HER2/neu were identified (10.4%, 9.9% and 9.5%, respectively; $p = 0.856$). Metastatic disease was demonstrated at the moment of diagnosis in 31 women (5.61%). In relation to the subtypes, metastasis was found more often in luminal A, HER2/neu and TN (35.4%, 22.5% y 22.5%, respectively; $p = 0.870$). The information on the distribution of pathological characteristics according to breast cancer subtypes and IHC classification appears in Table **2** and Figure **2**.

Most of the women underwent a conservative surgical procedure (64.49%, $p = 0.689$). In relation to the histological grade at the moment of diagnosis, the majority of the tumors were grade I (80.25%). In grade III tumors, a greater frequency of subtypes TN and luminal A was observed (13.2% and 8.8% respectively). As to systemic treatment (chemotherapy and/or hormonal therapy), 484 women (87.68%) received chemotherapy and 230 women received hormonal therapy (41.66%). In subtypes luminal A and TN, more subjects received chemotherapy (87.9% and

Table 1: Distribution of Clinical Characteristics According to Breast Cancer Subtypes Classified by Immunohistochemistry

N=552	Luminal A *n=182* (32.97%)	Luminal B *n=39* (7.07%)	Mixed *n=74* (13.41%)	HER2/neu *n=105* (19.02%)	Triple negative *n=152* (27.53%)	*p*
	n (%)	n (%)	n (%)	n (%)	n (%)	
Age ± SD	55.0 ± 11.9	52.3 ± 16.3	50.6 ± 12.0	51.3 ± 10.8	51.9 ± 13.5	0.044*
p¶	0.067	0.200	0.085	0.200	0.030	
≤ 40						
Yes	18 (9.8)	8 (20.5)	14 (18.9)	14 (13.3)	27 (17.8)	0.123
No	164 (90.2)	31 (79.5)	60 (81.1)	91 (86.7)	125 (82.2)	
p¶	0.084	0.202	0.166	0.911	0.098	
40-49						
Yes	48 (26.3)	7 (17.9)	24 (32.4)	32 (30.4)	45 (29.6)	0.539
No	134 (73.7)	32 (82.1)	50 (67.6)	73 (69.6)	107 (70.4)	
p¶	0.493	0.199	0.410	0.600	0.698	
50-59						
Yes	49 (26.9)	12 (30.8)	19 (25.7)	37 (35.2)	35 (23.0)	0.254
No	133 (73.1)	27 (69.2)	55 (74.3)	68 (64.8)	117 (77.0)	
p¶	0.726	0.684	0.648	0.070	0.129	
60-69						
Yes	42 (23.1)	7 (17.9)	11 (14.9)	16 (15.2)	29 (19.0)	0.264
No	140 (76.9)	32 (82.1)	63 (85.1)	89 (84.8)	123 (81.0)	
p¶	0.055	0.575	0.415	0.374	0.789	
> 70						
Yes	25 (13.7)	5 (12.8)	6 (8.1)	6 (5.7)	16 (10.5)	0.288
No	157 (86.3)	34 (87.2)	68 (91.9)	99 (94.3)	136 (89.5)	
p¶	0.323	0.798	0.443	0.047	0.610	
Menopausic state						
Yes	116 (63.7)	24 (61.5)	36 (48.6)	59 (56.2)	80 (52.6)	0.134
No	66 (36.3)	15 (38.5)	38 (51.4)	46 (43.8)	72 (47.4)	
p¶	0.071	0.512	0.083	0.676	0.127	

F: Frequency.
SD: standard deviation.
p: Pearson's chi squared test vs. Fisher's exact test.
*ANOVA.
¶intragroup analysis.



Luminal A: RE+ y/ó RP+ y HER2-, Luminal B: RE+ y/ó RP+ y HER2+, HER2/neu: RE-, RP- y HER2+, Mixte: RE+, RP+ y HER2+, Triple negative (TN): RE-, RP- y HER2-.

Figure 1: Age ranges and menopausic state according to breast cancer subtypes classified by immunohistochemistry.

Table 2: Distribution of Pathologic Characteristics According to Breast Cancer Subtypes Classified by Immunohistochemistry

N=552	Luminal A *n=182* (32.97%)	Luminal B *n=39* (7.07%)	Mixed *n=74* (13.41%)	HER2/neu *n=105* (19.02%)	Triple negative *n=152* (27.53%)	*p*
	n (%)	n (%)	n (%)	n (%)	n (%)	
Tumor size ≥ 2 cm						
Yes	164 (90.1)	37 (5.9)	67 (90.5)	95 (90.5)	137 (90.1)	0.092
No	18 (9.9)	2 (1.1)	7 (9.5)	10 (9.5)	15 (9.9)	
p[¶]	0.570	0.832	0.034	0.609	0.528	
Lymph node involvement						
Yes	64 (35.2)	16 (3.3)	34 (46.0)	40 (38.0)	72 (47.3)	0.257
No	118 (64.8)	23 (3.7)	40 (54.0)	65 (62.0)	80 (52.7)	
p[¶]	0.041	0.338	0.940	0.748	0.166	
Metastasis[§]						
Si	11 (6.0)	3 (0.5)	3 (4.0)	7 (6.7)	7 (4.6)	0.870
No	171 (94.0)	36 (6.5)	71 (96.0)	98 (93.3)	145 (95.4)	
p[¶]	0.778	0.497	0.790	0.625	0.517	

(Table 2). Continued.

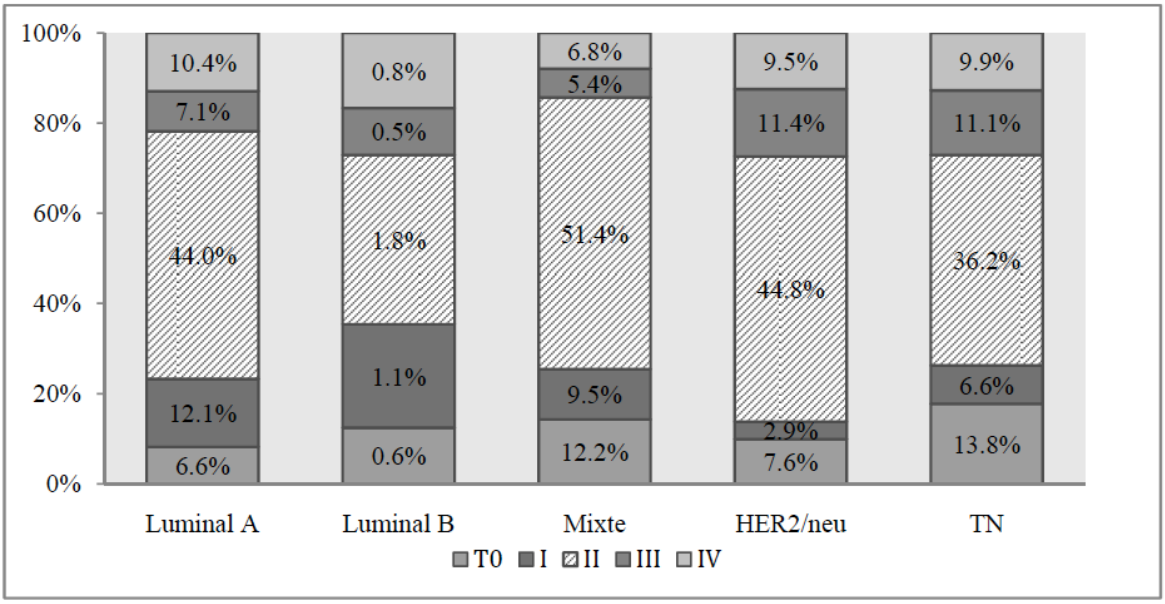
N=552	Luminal A *n=182* (32.97%)	Luminal B *n=39* (7.07%)	Mixed *n=74* (13.41%)	HER2/neu *n=105* (19.02%)	Triple negative *n=152* (27.53%)	*p*
	n (%)	n (%)	n (%)	n (%)	n (%)	
Stage						
0 (in situ)						
Yes	12 (6.6)	4 (0.6)	9 (12.2)	8 (7.6)	21 (13.8)	0.334
No	170 (93.4)	35 (6.4)	65 (87.8)	97 (92.4)	131 (86.2)	
p¶	0.040	0.561	0.708	0.283	0.143	
I						
Yes	22 (12.1)	7 (1.1)	7 (9.5)	3 (2.9)	10 (6.6)	0.020
No	160 (87.9)	32 (5.9)	67 (90.5)	102 (97.1)	142 (93.4)	
p¶	0.058	0.047	0.871	0.022	0.288	
II						
Yes	80 (44.0)	11 (1.8)	38 (51.4)	47 (44.8)	55 (36.2)	0.043
No	102 (56.0)	28 (5.2)	36 (48.6)	58 (55.2)	97 (63.8)	
p¶	0.335	0.050	0.083	0.373	0.147	
III						
Yes	13 (7.1)	3 (0.5)	4 (5.4)	12 (11.4)	17 (11.1)	0.401
No	169 (92.9)	36 (6.5)	70 (94.6)	93 (88.6)	135 (88.9)	
p¶	0.470	0.544	0.381	0.221	0.163	
IV						
Yes	19 (10.4)	5 (0.8)	5 (6.8)	10 (9.5)	15 (9.9)	0.856
No	163 (89.6)	34 (6.2)	69 (93.2)	95 (90.5)	137 (90.1)	
p¶	0.529	0.572	0.527	0.920	0.782	

F: Frequency.
p: Pearson's chi squared test vs. Fisher's exact test.
*ANOVA.
¶intragroup analysis.
§at the moment of diagnosis and/or during the evolution of the disease.

84.2%, respectively) and radiotherapy (82.4% and 89.5%, respectively). On the other hand, patients with luminal A subtype were treated more frequently with hormonal therapy (63.7%, p = 0.0001). A total of 25.72% (n=142) of the patients received adjuvant therapy.

At the moment of diagnosis 26.26% (n=145) of the patients had active disease. The most frequent subtypes were TN, HER2/neu and luminal A (30.9%, 28.6% and 23.1%, respectively). Persistent disease, defined as the presence of neoplastic illness after having received first-line adjuvant therapy, was observed in 30 subjects (5.43%; *p* = 0.06). Most of these cases with persistent disease had subtypes HER2/neu and TN (9.5% and 7.2%, respectively).

There was disease recurrence in 21.01% (n=116) of the patients. The subtypes with the greatest recurrence were; TN, luminal A and HER2/neu (28.3%, 19.8% and 19%, respectively). Finally, during the follow-up period (5 years), 4.34% (n = 24) of the patients died. Most of these deaths occurred in subtypes TN and HER2/neu (33.3% y 25%, respectively). The information on the distribution of characteristics related to treatment and outcome, according to the subtypes of breast cancer



Luminal A: RE+ y/ó RP+ y HER2-, Luminal B: RE+ y/ó RP+ y HER2+, HER2/neu: RE-, RP- y HER2+, Mixte: RE+, RP+ y HER2+, Triple negative (TN): RE-, RP- y HER2-.

Figure 2: Staging at the beginning of the disease according to breast cancer subtypes classified by immunohistochemistry.

Table 3: Distribution of Characteristics Related to Treatment and Outcome, According to the Breast Cancer Subtypes Classified by Immunohistochemistry

N=552	Luminal A *n=182* (32.97%)	Luminal B *n=39* (7.07%)	Mixed *n=74* (13.41%)	HER2/neu *n=105* (19.02%)	Triple negative *n=152* (27.53%)	*p*
	n (%)	n (%)	n (%)	n (%)	n (%)	
Surgery						
Radical	66 (36.3)	14 (35.9)	31 (41.9)	34 (32.4)	51 (33.6)	0.689
Conservative	116 (63.7)	25 (64.1)	43 (58.1)	71 (67.6)	101 (66.4)	
p¶	0.608	0.650	0.369	0.483	0.601	
Histological grade†						
I	153 (84.1)	34 (87.2)	57 (77.0)	82 (78.0)	117 (77.0)	0.352
II	13 (7.1)	3 (7.7)	6 (8.1)	15 (14.3)	15 (9.8)	
III	16 (8.8)	2 (5.1)	11 (14.9)	8 (7.7)	20 (13.2)	
p¶	0.244	0.403	0.395	0.244	0.418	
Chemotherapy						
Yes	160 (87.9)	34 (87.2)	66 (89.1)	96 (91.4)	128 (84.2)	0.278
No	22 (12.1)	5 (12.8)	8 (10.9)	9 (8.6)	24 (15.8)	
p¶	0.640	0.349	0.316	0.033	0.120	
Hormonal therapy						
Yes	116 (63.7)	25 (64.1)	45 (60.8)	22 (21.0)	22 (14.5)	0.0001
No	66 (36.3)	14 (35.9)	29 (39.2)	83 (79.0)	130 (85.5)	
p¶	0.0001	0.006	0.001	0.0001	0.0001	

(Table 3). Continued.

N=552	Luminal A *n=182* (32.97%)	Luminal B *n=39* (7.07%)	Mixed *n=74* (13.41%)	HER2/neu *n=105* (19.02%)	Triple negative *n=152* (27.53%)	*p*
	n (%)	n (%)	n (%)	n (%)	n (%)	
Adjuvant therapy						
Yes	28 (15.4)	7 (17.9)	32 (43.2)	54 (51.4)	21 (13.8)	0.0001
No	154 (84.6)	32 (82.1)	42 (56.8)	51 (48.6)	131 (86.2)	
p¶	0.0001	0.402	0.0001	0.0001	0.0001	
Radiotherapy						
Yes	150 (82.4)	35 (89.7)	59 (79.7)	86 (81.9)	136 (89.5)	0.133
No	32 (17.6)	4 (10.3)	15 (20.3)	19 (18.1)	16 (10.5)	
p¶	0.812	0.110	0.485	0.460	0.038	
Activity						
Yes	42 (23.1)	9 (23.1)	17 (23.0)	30 (28.6)	47 (30.9)	0.262
No	140 (76.9)	30 (76.9)	57 (77.0)	75 (71.4)	105 (69.1)	
p¶	0.067	0.948	0.509	0.528	0.136	
Persistence						
Yes	6 (3.3)	1 (2.6)	2 (2.7)	10 (9.5)	11 (7.2)	0.066
No	176 (96.7)	38 (97.4)	72 (97.3)	95 (90.5)	141 (92.8)	
p¶	0.064	0.715	0.417	0.062	0.563	
Recurrence						
Yes	36 (19.8)	6 (15.4)	11 (14.9)	20 (19.0)	43 (28.3)	0.215
No	146 (80.2)	33 (84.6)	63 (85.1)	85 (81.0)	109 (77.7)	
p¶	0.733	0.615	0.441	0.967	0.012	
Death						
Yes	5 (2.7)	1 (2.6)	4 (5.4)	6 (5.7)	8 (5.3)	0.580
No	177 (97.3)	38 (97.4)	70 (94.6)	99 (94.3)	144 (94.7)	
p¶	0.215	0.547	0.518	0.332	0.606	

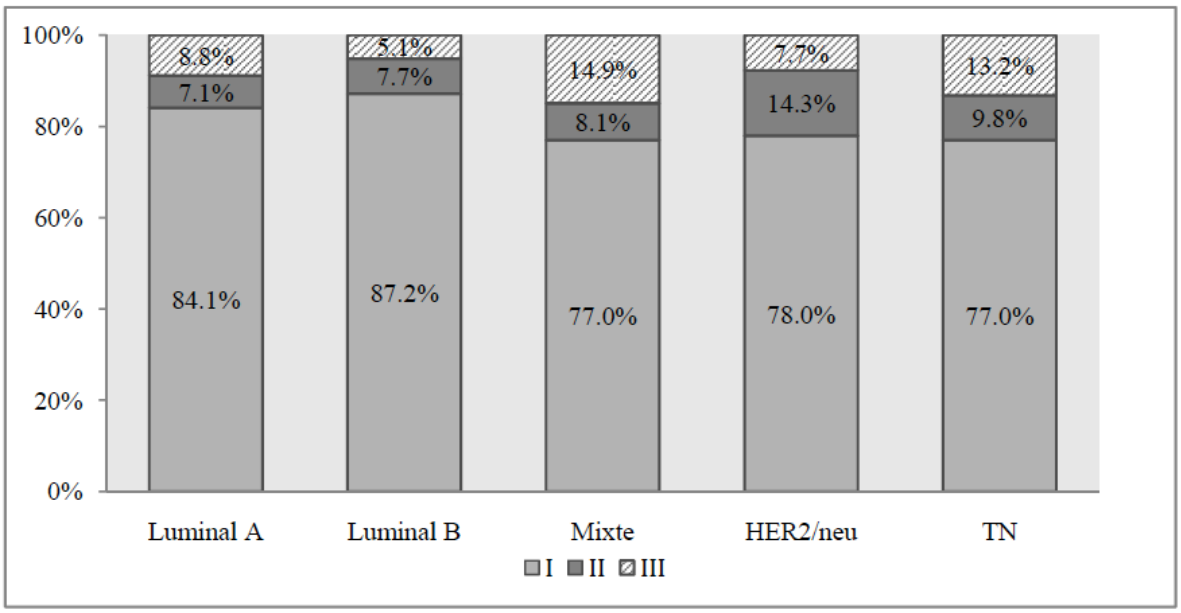
F: Frequency.
p: Pearson's chi squared test vs. Fisher's exact test.
*ANOVA.
¶intragroup analysis.
§at the moment of diagnosis and/or during the evolution of the disease.
+Scarff-Bloom-Richardson.

classified by IHC, are described in Table **3** and Figure **3**.

DISCUSSION AND CONCLUSIONS

This study identifies the distribution of breast cancer subtypes, using IHC, in a third-level hospital of northeastern Mexico. According to the profile, the most frequent subtypes were luminal A (32.97%), TN (27.53%) and HER2/neu (19.02%). These findings agree with a study done in 10 159 women, based on population registries in 12 hospitals in several countries (North America, Europe and Australia) from 1974 to 2005, where it was shown that the luminal A subtype was the most frequent (71.3%), followed by TN (16%) [31].

The triple negative subtype, with a 10 to 20% frequency in the majority of studies [32-36], was observed in over one-quarter of our study group (27.53%). This fact is relevant because of its direct relation with the prognosis, and never previously evidenced in our population. This subtype had a greater proportion of women < 40 years (17.8%; p = 0.098), a finding similar to, but higher than, the observations of Bauer and collaborators, who reported a TN frequency of 12.2% in Hispanic women [37]. In the case of subtype luminal A tumors, they showed



Luminal A: RE+ y/ó RP+ y HER2-, Luminal B: RE+ y/ó RP+ y HER2+, HER2/neu: RE-, RP- y HER2+, Mixte: RE+, RP+ y HER2+, Triplenegative (TN): RE-, RP- y HER2-, †Scarff-Bloom-Richardson.

Figure 3: Histological grade† of the disease according to breast cancer subtypes classified by immunohistochemistry.

some of favorable characteristics already demonstrated in previous studies (size less than 2 cm., differentiated tumors with low or moderate histological grade and incipient state) at the time of diagnosis [15]. However, subtype luminal B and mixed tumors showed a lower frequency of disease recurrence and persistence in our study, differing from previous studies where luminal A tumors had a worse prognosis than luminal B [38]. Our study coincides with others where breast cancer subtypes TN and HER2/neu have worse clinicopathologic and immunohistochemical characteristics than subtypes luminal A and B, associated with a greater frequency of disease recurrence and persistence [7]. The average age at diagnosis was 52 years, a finding similar to that observed in the Blows *et al.* study, who reported a rank between 50 and 59 years for all subtypes [31]. In Mexico, previous studies report similar figures [39-40]. Field *et al*., [41] report a high frequency of young women with subtype TN. Our study has similar results, where we identified a TN subtype in 29.6% if the women between 40 to 49 years of age. This finding is important, as these women still have reproductive age, many of them with young children. They are suddenly confronted with a disease that puts their family and emotional stability at risk, as well as a higher risk of physical disability and death.

Menopausic state had a higher association with subtype luminal A tumors, in more than 60% of the cases, agreeing with the information reported by Park and collaborators in a study done with women from Korea [42]. A high frequency of tumors ≥ 2 cm, lymphatic node involvement and metastasis was observed in the luminal A subtype, followed by TN; this data also agrees with previous studies, such as Park and collaborators' publication [42], however, it should be noted that a study done in Spain by Piñero-Madrona reports a higher frequency of lymph node involvement in the luminal B subtype, explained by the possible presence of a different immunophenotype in the breast tumors of their population [43]. As to the stage of the illness when first diagnosed, subtypes luminal A and TN showed the most advanced stages (III/IV) at the TNM staging in relation to the other subtypes. Subtype HER2/neu tumors also showed advanced stages, with 44% diagnosed at stage II and 11.4% at stage III, in agreement with the data published in Arrechea-Irigoyen and collaborators' study of a Spanish population [15]. In relation to the type of treatment, a radical mastectomy with chemoradiotherapy was the most frequent in luminal A and TN subtypes, principally associated with a more advanced stage of the disease and tumors with a more aggressive differentiation grading (II/III). However, despite the efforts to provide these patients with opportune treatment, it was in these subgroups where we observed higher frequencies of disease activity, persistence or recurrence. The differences observed in our study are comparable with studies done in other countries, and even more, our

results indicate that the use of these IHC markers in clinical environments allows for a better prognostic definition of breast cancer and for individual treatment designs, obtaining maximum benefit with the least possible risk.

One drawback to our study was that we did not have IHC tests that define the breast cancer basal subgroup (CK5/6, CK14 or p63). Although the terms "basal" and "triple negative" are on occasions used as synonyms, and share some similar clinical and prognostic characteristics, previous studies suggest that only between 60 [44] y 85% [45] of triple negative tumors have a basal/myoepithelial phenotype at the same time. The principal implication of this fact is that the basal subtype has the lower survival rate and recurrence time, which could partly explain the greater recurrence rate in the TN subgroup of our series [46].

Our institution's anatomical pathology service, in charge of assessing the IHC panel of the studied population, is widely recognized and accredited by external organisms, and their work and diagnostic quality is frequently evaluated. This reinforces the quality of the obtained data and minimizes the possibility of diagnostic error. The clinical and laboratorial information was obtained directly from the patients during their initial attention and from posterior assessments done at least every three to six months.

This study permitted the characterization of the immunohistochemical subgroups in breast cancer patients from cancer reference center in northeastern Mexico. It also allowed for the evaluation of the histological (intrinsic) subgroup distribution of infiltrating ductal breast cancer in relation to the principal clinical and pathological characteristics.

In conclusion, we can confirm the importance of assessing individual risk in each patient and identifying those patients with greater tumor aggressiveness, so that treatment can be provided during the early diagnostic phases. Likewise, those patients with less pathogenic tumors need not be subjected to the risks and secondary effects implicit in cytotoxic treatments.

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