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Breast carcinoma: Molecular classification using immunohistochemistry

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ABSTRACT
Objective: To classify different histological types for mammary carcinoma, invasive type, with the aid of immunohistochemistry. Methods: We selected 3033 positive cases, 2433 of the invasive type, which were classified into four groups: Luminal A, luminal B, HER2-eriched, and Basal like (triple negative). We conducted a retrospective observational study between the years 2011 and 2016.Results: 2062 (84.4%) of the related to CDI SOE, following the items in decreasing order: CLI (11.3); Invasive micropapillary carcinoma (1.11%) and other types with incidence less than 1%. Conclusion: This study allowed a histological differentiation and characterization of the IHQ profile of invasive mammary carcinoma over a 6 years in the Federal District.
DESCRIPTORS: Breast, Carcinoma, Cancer, Neoplasm, Immunohistochemistry.

RESUMEN
Objetivo: Clasificar diferentes tipos histológicos del carcinoma de mama de tipo invasivo, con ayuda de inmunohistoquímica. Métodos: Se seleccionaron 3033 casos positivos, de estos 2433 del tipo invasivo, los cuales fueron clasificados en cuatro grupos: Luminal A, Luminal B, HER2-eriched y Basal like (triple negativo). Realizamos un estudio observacional retrospectivo entre los años 2011 y 2016 Resultados: 2062 (84,4%) de los informes revisados estaban relacionados con CDI SOE, seguidos de los tipos en orden decreciente: CLI (11,3); carcinoma micropapilar invasivo (1,11%) y los otros tipos con una incidencia inferior al 1%. Conclusión: Este estudio permitió la diferenciación histológica y caracterización del perfil IHC del carcinoma invasivo de mama que se presentó en un período de 6 años en el Distrito Federal.
DESCRIPTORES: mama; Carcinoma, cáncer, neoplasia, inmunohistoquímica

RESUMO
Objetivo: Classificar diferentes tipos histológicos para carcinoma mamário, do tipo invasivo, com auxílio da imuno-histoquímica. Métodos: Selecionamos 3033 casos positivos, destes, 2433 do tipo invasivo que foram classificados em quatro grupos: Luminal A, luminal B, HER2-eriched e Basal like (triplo negativo). Realizamos um estudo retrospectivo observacional entre os anos de 2011 e 2016Resultados: 2062 (84,4%) dos laudos revisados, foram referentes a CDI SOE, seguido pelos tipos em ordem decrescente: CLI (11,3); carcinoma micropapilar invasivo (1,11%) e os demais tipos com incidência menor que 1 %. Conclusão: Este estudo permitiu a diferenciação histológica e caracterização do perfil IHQ do carcinoma mamário invasivo ocorridos em um período de 6 anos no Distrito Federal.
DESCRITORES: Mama; Carcinoma, Câncer, Neoplasia, Imuno-histoquímica.

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INTRODUCTION

Breast cancer represents one of the main public health problems in the world. Except for the skin, it is the most common neoplasm among women. In 2008, about 13,7% of cancer deaths worldwide were due to breast cancer (Gebrim[7], 2015). In Brazil, this neoplasm is the main cause of death among women. In 2010, the Ministry of Health estimated approximately 49.240 new cases in the country, being more common in post-menopausal patients, aged between 50 and 69 years (Cintra[3], 2012). It is a heterogeneous disease with different histological, molecular and clinical presentations (Vinay K, et al. [18] 2010).

The diagnosis and characterization of breast cancer begins with the morphological evaluation by conventional histopathology. Briefly, after removing the suspect tissue, it is processed and cut into thin slices of 3 to 5 microns, mounted on slides that are stained with hematoxylin and eosin. These slides are examined by the pathologist who determines whether there is malignancy in the sample and, if so, performs the histological classification of the neoplasia. The World Health Organization (WHO) predicts several histological types of breast cancer (Lakhani SR, et al. [10] 2012.), the most common being the so-called invasive breast carcinoma, with no special type (infiltrating ductal carcinoma, without other specifications / CDI-SOE) with about 5080% of the cases. Then there is the invasive lobular carcinoma (ILC), which represents 5 to 15% of the cases (Table 1).

After the conventional histopathological examination is completed, it is necessary to research the expression of the Estrogen Receptor (ER), Progesterone Receptor (PR) and the protein of the HER2 / neu gene (HER2). The most widespread method for this purpose is immunohistochemistry (IHC), which allows to determine in situ, that is, in the tissue itself, protein expression in the context of tumor morphology. The routine use of IHC for the analysis of ER, PR and HER2 expression provides critical information on prognostic and predictive factors for breast carcinomas and will

TABLE 1 - Prevalence of histological types of breast carcinomas recognized by the WHO

Tipo Histológico	Prevalência (%) ROSEN (2001)	Prevalência (%) ELLIS (2003)	Prevalência (%) RAKHA (2006,2008)	Prevalência (%) LOUWMAN (2007)
CDI-SOE	65-80	50-80	56.4	78
CLI	5	5-15	15	11.1
Ca. tubular	<2	<2	4.4	2.2
Ca.cribriforme	<4	0,8-3,5	0,6	0,3
Ca. micropapilar invasivo	<2,7	<2	-	-
Ca. mucinoso	<2	2	1.4	2.2
Ca. metaplásico	<5	<1	-	0,2
Ca. com características medulares	<5-7	1-7	2,6	1,1
Ca. apócrino	<1-4	<4	-	-
Ca. com células e anel de sinete	-	-	-	-
Adenomioepitelioma com carcinoma	-	-	-	-
Ca. adenoide cístico	-	0,1	-	0,1
Ca. com diferenciação neuroendócina	-	-	-	-

Ca. secretório	-	<0,15	-	-
Ca. papilar invasivo	-	-	-	-
Ca. de células acinares	-	-	-	-
Ca. muco-epidermóide	-	-	-	-
Ca. polimórfico	-	-	-	-
Ca. oncocítico	-	-	-	-
Ca. rico em lipídeos	<1	<1-6	-	-
Ca. rico em glicogênio	<1-3	1-3	-	-
Ca. sebáceo	-	-	-	-

Rosen, P.P., (2001); Ellis, P., Schnitt, S.J., Sastre-Garau, X., et al.(2003); Rakha, E.A., El-Sayed, M.E., Menon, S., Green, et al. (2008); Rakha, E.A., Putti, T.C., Abd El-Rehim, (2006).

guide subsequent therapy (Rakha EA, et al.[14] 2010).

From the 2000s, the works led by Perou and Sorlie (Perou CM, et al.[11] 2000); (Sorlie T, et al.[15] 2001); (Sorlie T, et al.[16] 2003) using a gene expression profile (GEP), he identified 5 molecular subtypes of breast carcinoma: luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like. Subsequently, it was found that each molecular class correlates with different survivals and responses to chemotherapy (CTX). Several other classifications were proposed, but the groups Luminal A, Luminal B, HER-2-enriched and Basal-like were constant.

In summary, the molecular subtype Luminal A (LumA), is characterized by the similarity that neoplastic cells present with normal cells found in the mammary ducts. This subtype represents approximately 60% of breast tumors, and has a better prognosis. Most of them are smaller than the others, and are characterized by high expression of ER, with low proliferative index, and low histological grade. It is the group with the best benefit for hormone therapy, and limited benefit for CTX (Cirqueira[4],2011).

Luminal B (LumB) tumors, like Luminais A, have ER expression. And they differ from those due to the high proliferative index, and association with the expression of HER2. They have a higher proliferation rate, and their prognosis is worse when compared to the others. LumB tumors have a higher recurrence rate and lower survival. Better response to CTX and worse response to hormone therapy is expected (Cirqueira[4],2011).

As stated above, Luminal A and Luminal B tumors are characterized by the expression of hormone receptors, mainly ER. The other tumor types, characterized by the absence of expression of these receptors are HER2-enriched and Basal-Like. HER2-enriched is characterized by the high expression of the HER2 / neu gene and other related genes. Generally, these tumors are poorly differentiated and have a high proliferative rate, being associated with a high frequency of brain metastasis. Patients with this classification have intrinsic resistance to endocrine therapies, but benefit from antiHER2 therapy and traditional CTX (Cirqueira[4],2011).

Finally, Basal-Like tumors comprise about 13% to 25% of breast tumors. This subgroup is negative for hormone receptors and HER2, high histological grade, high proliferation rate and are associated with lower patient survival. Represent a group of tumors without specific treatment, but chemosensitive (Vinay K[18], et al. 2010).

The contribution of methods using a gene expression profile was unmatched for understanding breast cancer, however the use of these methods is not practical from an economic or technical point of view, requiring specialized laboratories at high cost. As a result, several researchers have proposed that the IHC panel can replace molecular methods (Tang P; Tse GM17. 2015). In 2013, the St. Gallen convention recommended a molecular classification of breast cancer based on IHC (Goldhirsh A, et al8. 2013). This classification uses a combination of the results for RE, RP, HER2 and Ki67 (Table 2).

The present work aims to correlate the histological subtypes of breast carcinomas with the molecular types defined by the IHC.

METHODS

A retrospective and observational study was carried out with a review of 3,033 reports of breast IHC at a pathological anatomy laboratory in Brasília-DF (Ladeira Laboratory) in the years 2011 to 2015. Cases of neoplasia "in situ" were excluded from the analysis in order to to preserve only the cases of invasive neoplasia, totaling 2433 cases analyzed.

The cases were morphologically evaluated by conventional histomorphology and the immunohistochemistry reaction was revealed with the NOVOLINK biotin-free system (Leica-Biosistems). In the search for hormone receptors, the following monoclonal antibodies were used: RE clone 6F11 (LeicaBiosistems) at a dilution of 1: 400; RP clone 16 (LeicaBiosistems) at 1:800 dilution. The grading of hormone receptors was performed using the Allred method (Allred DC, et al. 1998) The Allred score assesses the proportion of positive cells along with the intensity of the reaction. The score ranges from 0 to 8, with cases greater than 3 being considered positive.

The antibody used to demonstrate HER2 expression was SP3 (Epitomics) at a dilution of 1: 500. The evaluation of HER-2 expression followed the guidelines of the American Association of Clinical Oncology and the American College of Pathologists (ASCO/CAP) (Wolff AC, et al[20]. 2013). The cases identified in the immunohistochemistry as doubtful

were referred for genetic amplification research by fluorescence in situ hybridization (FISH).

The antibody used for Ki67 was SP6 (Cell Marque) at a 1:500 dilution. The reaction was read manually by recording the proportion of positive cells for the label.

Based on the information contained in the reports, each case received a molecular classification based on the proposal by StGallen 2015 (TABLE 2).

The histological subtypes were then correlated with the molecular subtype. Microsoft Excel 2013 software was used for data processing.

RESULTS

Of the total of 3.033 reports reviewed, 2433 were related to invasive carcinoma and contained all the information to be included in the study. The most common histological type was infiltrating ductal carcinoma, NST (invasive breast carcinoma without a special type in the WHO classification 2012) totaling 2062 cases (84.4%). The other histological types in decreasing order were: invasive lobular carcinoma (11,3%), invasive micropapillary carcinoma (1,11%) and with a prevalence of less than 1% we observed mucinous carcinoma, tubular carcinoma, invasive ductal and lobular carcinoma, papillary carcinoma invasive, apocrine carcinoma, metaplastic carcinoma, tubulo-lobular carcinoma, adenoid-cystic carcinoma, carcinoma with medullary characteristics, ductal carcinoma with neuro-endocrine differentiation, secretory carcinoma and cribriform carcinoma.

Table 3 lists the histological types with the respective molecular classification by IHC. For NST cases, it is observed that lower histological degrees were related to a higher proportion of LumA cases. For example, NST grade 1 cases, presented a proportion of LumA cases of 92,7%, CDI-SOE grade 2 cases, the proportion of LumA cases was 54,9% and for grade 3 only 6,9%. Invasive micropapillary carcinoma was mostly classified as LumB. Mucinous carcinoma had 83% of the cases classified as LumA. Tubulo-lobular and cribriform carcinoma had all cases classified as LumA. Adenoid-cystic carcinoma had all cases classified as TNBC. Most cases of metaplastic carcinoma were classified as TNBC.

TABLE 2. Molecular classification of breast cancer based on IHC. Proposed by the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer (Coates AS, et al. 2015).

TIPO MOLECULAR	CRITERIO
Luminal A (LumA)	RE+ e /ou RP+ HER2 – Ki67% baixo*
Luminal B HER2- (LumB HER2-)	RE+ e /ou RP+ HER2 – Ki67% alto*
Luminal B HER2+ (LumB HER2+)	RE+ e /ou RP+ HER2 +
HER2-neu non-luminal (HER2 N/L)	RE -/ RP- HER2 +
Triple Negative Breast Cancer (TNBC)	RE- / RP- HER2 -

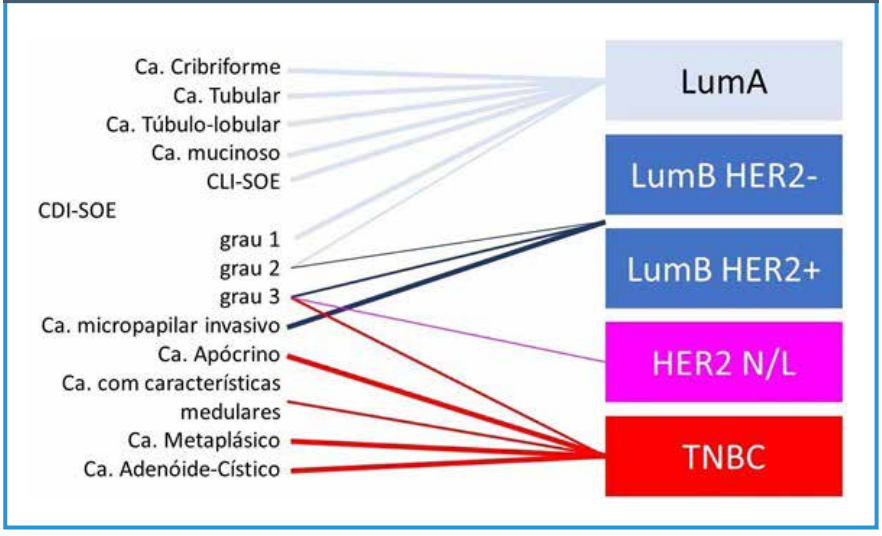
* To define the cutoff point for Ki67, the median expression was calculated in cases positive for RE (20%). Values ≤ 20% were considered low, values> 20% were considered high.

TABLE 3

Tipo Histológico	LumA	LumB/ HER2-	LumB/ HER2+	HER2 N/L	TNBC	Total	(%)
CDI-SOE	975 (47,3%)	492 (23,9%)	186 (9%)	143 (6,9%)	266 (12,9%)	2062	84,44%
grau 1	280(92,7%)	11(3,6%)	7(2,3%)	1 (0,3%)	3 (1,0%)		
grau 2	647(54,9%)	297(25,2%)	110 (9,3%)	62(5,3%)	63(5,3%)		
grau 3	48(6,9%)	266(38,1%)	81(11,6%)	83(11,9%)	221(31,6%)		
CLI-SOE	213(76,6%)	53(19,1%)	7(2,5%)	0	5 (1,8%)	278	11,38%
Ca. micropapilar invasivo	5(18,5%)	16(59,3%)	3(11,1%)	1 (3,7%)	2 (7,4%)	27	1,11%
Ca. mucinoso	20(83%)	3(12,5%)	1(4,2%)	0	0	24	0,98%
Ca. invasivo ductal e lobular	3(33.3%)	3(33.3%)	1 (11,1%)	1 (11,1%)	1 (11,1%)	9	0,37%
Ca. tubular	9 (100,0%)	0	0	0	0	9	0,37%
Ca. papilar invasivo	4(66,7%)	2(33,3%)	0	0	0	6	0,25%
Ca. Apócrino	1(16,7%)	1(16,7%)	0	1(16,7%)	3 (50,0%)	6	0,25%
Ca. Metaplásico	0	1(14,3%)	0	0	6(85,7%)	7	0,29%
Ca. Túbulo-lobular	3(100,0%)	0	0	0	0	3	0,12%
Ca. Adenóide-Cístico	0	0	0	0	3(100,0%)	3	0,12%
Ca. com caract. medulares	0	1 (50,0%)	0	0	1(50,0%)	2	0,08%

Ca. com dif neuroendócrina	2(66,6%)	1(33,3%)	0	0	0	3	0,12%
Ca. Secretor	0	1(100,0%)	0	0	0	1	0,04%
Ca. Cribriforme	2(100,0%)	0	0	0	0	2	0,08%

Figure 1 Distribution of histological types according to the molecular classification. The line thickness indicates the proportion of cases related to a given classification



DISCUSSION

In this study, we correlated several histological types of breast carcinoma with their molecular classification, through immunohistochemistry. The evaluation of the histological type associated with the search for expression of hormone receptors, HER2 and Ki67 are prognostic and predictive factors taken into account in the management of the most appropriate therapies for each tumor type.

The most common histological type observed in this study was invasive ductal carcinoma, without other specifications (NST) (Invasive breast carcinoma without a special type in the WHO classification 2012). We note the fact that these tumors are not a homogeneous group, but that they can be better classified according to the histological grade. Grade 1 NST were mostly classified as LumA. The NST grade 2, behaved as the most heterogeneous histological type in the sample. Half of the cases (54,9%) were classified as LumA, with the remaining cases distributed by the LumB (34.5%) HER2 N/L (5%) and TNBC (5%) groups. Grade 3 NOS carcinomas had a low prevalence of LumA cases, with most cases distributed between LumB, HER2 N/L and TNBC. This fact demonstrates that the histological grade is capable of synthesizing factors of tumor biology, resulting in different molecular classifications.

The second most common histological type, infiltrating lobular carcinoma, was predominantly classified as LumA. Some histological types had all cases classified in the same molecular group. Adenoid-cystic carcinoma had all its cases classified as TNBC. Cribriform, tubular and tubule-lobular carcinomas were all classified as LumA. The cases of invasive micropapillary carcinoma were predominantly LumB type. Mucinous carcinomas were predominantly LumA (83%) but a small group was classified as LumB HER2- (12,5%).

In a similar approach, which used IHC for molecular classification of histological subtypes, Alvarenga et al. (Alvarenga CA, et al. 2012) reached results similar to those presented here. Studying a previously selected group of special subtypes, it was found that the tubular subtype belonged to the LumA classification. The mucinous subtype was distributed in LumA and LumB. The metaplastic and medullary subtypes were predominantly basal-like (TNBC in the classification of the present study). Finally, the micropapillary subtype showed a predominant LumB pattern.

Weigelt and cols[19] (2008), in a study involving immunohistochemical and transcriptomic analysis of several molecular subtypes, they found that some histological subtypes are specific entities with their own patterns of molecular expression and transcriptional activity. It was found that at the transcriptomic level, the histological subtypes are more homogeneous with each other than the NST. The tubular and mucinous histological subtypes showed a clear luminal pattern while the adenoid-cystic, medullary and metaplastic pattern expressed a basallike phenotype. In the present study, it was possible to reproduce this finding, characterizing the NST subtype, mainly grade 2, as a heterogeneous tumor class, with wide distribution by molecular types.

Figure 1 presents a summary of the correlations between the histological and molecular subtypes observed in this study, which reproduces the findings in the literature. We have that low grade tumors (cribriform, tubukar, tubule-lobular, mucinous, NST and ILC-NOS grade 1) are predominantly classified as LumA. Invasive micropapillary carcinoma is strongly related to the molecular type LumB. Several special types are classified as TNBC (Apocrine carcinoma, with medullary, metaplastic and adenoid-cystic characteristics). The NST grades 2 and 3 are very

heterogeneous, but the proportion of LumA cases decreases as the histological grade increases.

CONCLUSION

In general, this study demonstrated the high prevalence, in female patients, of invasive breast carcinoma without other specifications (NST), followed by infiltrating lobular carcinoma without other specifications (ILC-NOS) as well as, the other types histological findings that also presented results similar to those already described in the literature.

In this context, the routine use of immunohistochemistry allowed, in a practical and economical way, from the expression of ER, PR and HER2, the molecular classification as proposed by St. Gallen in 2013. It was also possible to demonstrate the heterogeneity of the disease, and that the knowledge about prognostic and predictive factors, directly implies the management of more appropriate therapies, with less adverse effects.

Through this work we can perceive the importance of IHC in diagnosing the molecular subtype, providing the necessary knowledge for individualized treatments, based on new molecular knowledge, however, new studies are necessary in order to determine future therapeutic targets in the different histological types of breast carcinoma. ■

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