Ductal invasive mammary carcinoma - clinicopathological prognostic factors related to immunohistochemical expression of hormonal receptors and Her2/neu oncoprotein

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CRAIOVA 2011
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INTRODUCTION
Breast cancer is a multifactorial disease, consisting of distinct biological subtypes, with different natural evolution and a broad spectrum of clinical, pathologic and molecular characteristics, with different prognosis and therapeutic implications. Due to the disease heterogeneity there is a relentless pursuit in identifying certain predictive markers concerning disease prognosis and treatment response. At present breast cancer is regarded as a unique disease in oncology and the specific markers - ER, PR and HER2/neu are used to predict the treatment response to guide the therapeutic plan.

STUDY OBJECTIVES
The aim of the study was to estimate the clinical-pathological parameters wich characterize the study group and focused on comparative investigation of hormonal receptors (estrogen receptor ER and progesterone receptor PR) and Her2/neu oncoprotein expression, according to which we ranked the cases into molecular classification subtypes, determining certain correlations between them and morpho-clinical prognostic factors. The cases were also analysed regarding the proliferative activity quantified by Ki67 index as a prognostic marker and subsequently correlated with clinico-morphological studied parameters.

ESTROGEN CARCINOGENESIS
One of the most important risk factors in breast cancer is the estrogen exposure. The metabolism of estrogen to genotoxic, mutagenic metabolites and the stimulation of tissue growth explain the process of mammary carcinogenesis induced by estrogen, following the sequence: initiation, promotion and progression of carcinogenesis. The knowledge of the central role of the estrogen in breast cancer has already led to the molecular classification of breast cancer by the hormonal receptor derived gene expression profile. [187]
MATERIAL AND METHODS

A. Investigated material.

The studied group consisted of 380 cases of invasive ductal mammary carcinomas type NOS, surgically treated between 2005-2009 in County Emergency Clinical Hospital of Craiova.

B. Methods

Histopathological study. The surgically specimens were fixed in 10% buffered formalin and processed by paraffin-embedded and were analysed regarding the tumor size, tumor grade, nodal status, vascular and perineural invasion, preinvasive lesions associated, distant metastases and pTNM classification.

Immunohistochemical study included 75 selected cases and the method used was the indirect method in two steps, using EnVision technique of polymeric amplification. The antibodies used were: estrogen receptor (1D5 clone, DAKO), progesterone receptor (PgR 636 clone), Her2/neu oncoprotein (policlonal, DAKO) and Ki67 (MIB-1 clone, Dako).

Statistical Analysis used correlation coefficient (Pearson) and comparative tests averages (test t Student). To assesses the dependence between two factors we realised incidence tabels which were evaluated by means of Chi square test.

RESULTS

VIA. Clinico-morphological study results

Between 2005 and 2009 we noticed an increasing incidence, from 57 cases in 2005 to 95 cases in 2009. Most of them were female, only one case was diagnosed to a male patient. 35% were diagnosed at women aged between 50 and 60 and 28% were diagnosed at women between 61 and 70. Low incidence was noted under 30 years old (1%) and after 80 years old (2,6%). 42,9% of cases were classified in T2 category and 36,3% in T1, depending on the tumour size. From the point of view of differentiation degree, the most frequent tumours were low-differentiated (G3), with a percent of 78% of cases, followed by G2 (19%) and G1 (3%). The lymph node excision was performed for 327 patients and we obtained the follow results: 26,3% pN0, 18,3% pN1, 19,9% pN2, 27,8% pN3 and 7,7% pNx.
56 cases presented tegument invasion. 113 cases associated vascular invasion and 118 cases associated perineural invasion and 21 cases presented distant metastases.

According to these results, we ranked the cases in evolution studies: stage I: 16.9%, stage II: 24.7%, stage III: 51.7% and stage IV: 6.5%.

**Statistical Analysis.** Chi square test indicated a significant association between: age and tumoral stage (p<0.001), tumoral size and tumoral stage (p<0.001), lymph node status and tumoral stage (p<0.001) and tumoral stage (p<0.001), tumoral grade and vascular invasion (p<0.05), tumor size and vascular invasion (p<0.001), perineural invasion (p<0.001) and tegument invasion (p<0.001).

**VI B. IMMUNOHISTOCHEMICAL STUDY RESULTS**

**Immunohistochemical study.** All the 75 cases were evaluated immunohistochemically, in terms of hormonal receptors expression, determining the tumour immunophenotype at the same time. The majority of these 75 cases, representing a percent of 73.3% were ER+, the rest of 26.7% being ER-. As far as progesterone receptor expression is concerned, 62.6% of cases were PR+ and only 37.4% were PR-. Determination of tumour immunophenotype (corroboration between hormonal receptors expression ER and PR) allowed the classification of mammary tumours, in decreasing order of frequency, into the following categories: ER+PR+ (58.6% of cases), ER-PR- (22.8%), ER+PR- (14.6%) and ER-PR-(4% of cases).

Her2/neu oncoprotein expression was also analysed in all the 75 mammary carcinomas in our study and we determined a negative immunoreaction of score 0 in 53 cases (70.6%) and score 1+ in 10.7% of the cases; the tumours of score 0 and score 1+ were in a percent of 81.3%. Her2/neu expression indicated a score 2+ (equivocal immunohistochemical stain) in 3 cases (4%) and score 3+ (positive immunohistochemical stain) in other 11 cases (14.8%).

We focused on the relationship between hormonal receptors and Her2/neu status and subsequently correlated the results with the studied clinical and morphoclinical parameters. We determined that 9.5% of cases with positive Her2/neu of score 3+ were also positive for both hormonal receptors. On the contrary, 29.5% of the cases with positive Her2/neu were characterized by the absence of nuclear stain for both hormonal receptors. 34% of cases Her2/neu positive of score 3+ presented PR+ şi ER-, and 9.2% of the cases Her2/neu + associated ER+ and PR-. We determined a
positive Her2/neu immunohistochemical stain of score 3+ in the tumours with negative hormonal receptors (5 cases, 29,5%) and the absence of Her2/neu expression (Her2/neu score 0-1) in the tumours with positive hormonal receptors (39 cases, 88,5%).

Analysis of the relationship between the response to hormonal receptors and Her2/neu status allowed the distribution of 72 cases into molecular classification, using “surrogate immunohistochemical criteria”. 3 cases (4%) with equivocal immunohistochemical stain (Her2/neu score 2+) were excluded. Thus, we obtained the following incidences of molecular subtypes: luminal A subtype, with the greatest incidence – 70% of cases, followed by basal subtype with 14,7%. Luminal B subtype represented 8,3% of cases and HER2 subtype had the lowest incidence, respectively 7% of cases.

We analysed the correlations obtained between molecular subtypes and morphoclinical parameters and we determined that luminal A subtype characterized the groups aged over 60 (p<0,05). The majority of cases belonging to luminal B were aged under 60 (p<0,05). Regarding HER2 cases, 40% were met at patients aged under 50 and 60% at the group aged 51-60. There was no case over the age of 60. The basal group was met in 90% of the cases aged over 50(p<0,05).

We investigated the relationship between molecular subtypes and tumour dimension and we observed that tumours of small dimensions (T1-T2) prevailed (92% of the cases) in luminal A. Regarding luminal B subtype, 83,4% of cases had dimensions classified in T2-T3. Tumours in the subtypes with negative hormonal receptors had large dimensions specific to T2-T3 (60% of HER2 tumours, respectively 91% of basal subtype).

As concerns tumour differentiation degree, luminal A subtype was associated in percent of 58% of cases with well and moderately differentiated tumours. Luminal B subtype presented low differentiated tumours in 66,7% of the cases. HER2 subtype was associated in 80% of cases with low-differentiated tumours G3. Basal subtype presented a moderate and low differentiation degree in 91% of cases.

The Ki67 index exhibited that 42,6% of cases had low proliferative activity, 32% of cases had intermediate proliferative activity and 25,4% of cases had higher proliferation.

62% of luminal A subtype presented a low proliferative activity, unlike luminal B subtype which presented in 66,6% of cases high proliferative capacity. Non-luminal
subtypes presented higher values of prolifertive index (100% HER2 subtype and 90.9% basal subtype had index Ki67> 15%). Reporting Ki67 index to molecular subtypes were very high significant (p<0.001).

DISCUSSION

The correct treatment of breast cancer is a multidisciplinary treatment, the sequence of therapeutic methods and their aggressiveness being conditioned by the histopathologic type, tumour dimensions, adenopathies, the patients’ age and their menopause status. The specific markers ER, PR and Her2/neu are used in treatment response prognosis and in guiding the therapeutic plan.

The majority of patients in the study group were aged over 50 (82.8%). Most tumours had large dimensions, 48.9% of the cases were categorized in T2 and T3. 78% of cases were low-differentiated, classified in G3 category, comparatively with only 3% of cases of well differentiated tumours G1. The histologic grade and the nuclear grade are prognostic factors useful for the stratification by stage, especially for cases without lymph node metastases. [147]

Evaluation of hormonal status determined that 73.4% of cases in our studied group were ER+ and 26.6% ER-, respectively 62.6% were PR+ and 37.4% PR-, results which can be compared with those obtained by large studied groups and published in the specialty literature. [62]

Analysing the incidence of immunophenotypes, we observed that most cases belonged to phenotype ER+PR+, 44 cases (58.6%), followed by phenotype ER-PR- with 17 cases (22.8%). 11 cases (14.6%) were ER+PR-, and the lowest incidence was determined at phenotype ER-PR+ which registered 3 cases (4%). In the study carried out by Rakha et. al. these immunophenotypes had the following frequency: ER+PR+ 55.3%, ER+PR- 15.6%, ER-PR+ 3.4% and ER-PR- 27%. [62]

In our study, only 11 cases (14.8%) expressed positive Her2/neu immunohistochemical stain scored 3+. Lisa Ryden et. al. [157] shows that over-expression or Her2 gene amplification is identified in 10-30% of primary mammary carcinomas and it is correlated with an aggressive tumour subtype and with short follow-up survival.
The studied correlations between hormonal receptors expression and Her2/neu status led us to make an association between the cases with Her2/neu negative scored 0-1 and the cases in which both hormonal receptors were positive (88.5% for ER+PR+ phenotype); the cases with positive Her2/neu scored 3+ were correlated with cases with negative hormonal receptors (29.5% for ER-PR-, respectively 34% for ER-PR+ phenotype). Our results comply with most of the studies in specialty literature and show that the presence of oestrogen receptor ER and progesterone receptor PR is inversely correlated with Her2 expression. [11]

We categorized the cases into molecular classification. Most of the cases in our study were ranked in luminal A (70%), followed by basal (14.7%). Luminal B had an incidence of 8.3% and HER2 had the lowest incidence, 7% of the cases. Large studies in specialty literature reported similar results [182].

Specific literature show that tumors in the HER2 and basal subtypes were more frequently high grade and larger, and they occurred in younger patients compared with those in the luminal A subtype. Tumors overexpressing HER2 were statistically significantly more likely to be multicentric/multifocal, have a high nuclear grade and have lymphvascular invasion than luminal A tumors. The luminal B subtype present characteristics that fell between those of luminal A and HER2 tumors subtypes. [182]

Regarding the proliferativ index Ki67, our results are comparable with other studies, which show that a higher Ki67 index is correlated significant with young age, large tumors, positive lymph nodes, negative ER/PR and supraexpression Her2. On the other hand, a lower Ki67 index correlated with a favourable prognosis and a late recurrence. [146]

**CONCLUSIONS**

- Our study revealed a rising incidence, from 57 cases in 2005 to 95 cases in 2009.
- High incidence (35%) was noted between 50 and 60 years old, with a strong statistical association with the stage of disease (p<0.01)
- 42.9% of cases were classified in T2 category.
- The most frequent tumors were low-differentiated (78%), significant associated with the age of the patient (p<0.05).
- 27.8% of cases were pN3. There was a significant association between lymph node status and tumoral size and tumoral stage (p<0.001).
- 73.3% of cases were ER+ and 62.6% of cases were PR+.
- 14.8% of cases were Her2/neu positive of score 3+ and 81.3% of cases were negative of score 0 and 1+. Her2/neu positive cases were associated with ages under 40 years old and T2-T3 tumors (p<0.001).
- Luminal A subtype (70%) was associated with ages over 60 and T1-T2 tumors (p<0.05). 58% of cases were G1-G2 tumors. 62% of cases presented high proliferative activity.
- Luminal B subtype (8.3%) was associated with ages under 60 and T2-T3 tumors (p<0.05). All the cases were G2-G3 tumors. 66.6% of cases presented high proliferative activity.
- HER2 subtype (7%) was associated with ages under 60 and T2-T3 tumors (p<0.05). 80% of cases were G3 tumors. All the cases presented high proliferative activity.
- Basal subtype (14.7%) was associated with ages over 50 and T2-T3 tumors (p<0.05). 91% of cases were G2-G3 tumors. 90.9% of cases presented Ki67 index > 15%.
REFERENCES


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PERSONAL PAPER WORKS IN PHD THESIS THEME

Published paper works

- **Felicia Recareanu**, Cristiana Simionescu, Claudia Georgescu, Claudiu Margaritescu, Elena Pirici.Ductal Invasive Mammary Carcinoma-Clinicopathological prognosis factors related to immunohistochemical expression of hormonal receptors and Her2/neu oncoprotein – to be published

Paper works presented at Congresses and Conferences

- **Felicia Recăreanu**, Cristiana Simionescu, Claudia Georgescu, Nicoleta Pătrana. Molecular classification of ductal invasive mammary carcinoma


