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

FELICIA RECĂREANU1), CRISTIANA SIMIONESCU2), CLAUDIA VALENTINA GEORGESCU3), ELENA PIRICI4)

1) PhD candidate
2) Department of Pathology
University of Medicine and Pharmacy of Craiova
3) Emergency County Hospital, Craiova
4) Emergency County Hospital, Ramnicu Valcea

Abstract
We analyzed 75 cases of invasive ductal mammary carcinoma type NOS 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 in molecular classification subtypes, determining certain correlations between them and morphoclinical prognostic factors. 73.4% of cases were ER+ and 26.6% were ER-. PR was present in 62.6% of cases and absent in 37.4%. Phenotype ER+PR+ (58.6%) had the highest incidence, followed by ER-PR- (14.6%) and ER+PR- (14.6%). Phenotype ER-PR+ (4%) registered the lowest incidence. 14.8% of tumors were Her2/neu + score 3+, 4% had equivocal score 2+ and 81.3% were negative Her2/neu scored 0 and 1+. 9.5% of cases Her2/neu positive scored 3+ were ER+PR+ and 88.5% of cases Her2/neu negative scored 0–1 were ER+PR+. In terms of the correlation among the status of ER, PR and Her2/neu, we determined a molecular classification of the cases, obtaining the following incidences: luminal A 70% of cases, basal 14.7% of cases, luminal B 8.3%, the lowest incidence being registered at Her2, 7% of cases. Luminal A and basal subtypes were associated with patients aged over 50 years (82% for luminal A and 90% for basal), whereas luminal B and Her2 subtypes were registered mostly at patients aged under 60-year-old (83% for luminal B and 100% for Her2). Luminal A subtype was characterized by small tumors (92% of cases were T1–T2), well and moderately differentiated tumors (58% of cases were G1–G2). 83.3% of cases in luminal B subtype had tumors with dimensions ranked T2–T3, all cases being moderately and low differentiated. Her2 subtype had T2–T3 tumors in 60% of cases, which were G3 low differentiated in a percent of 80%. The basal subtype mostly had tumors larger than 5 cm (91% of cases were T2–T3), out of which only a case (9%) presented well-differentiated G1.

Keywords: mammary carcinoma, prognosis, hormonal receptors, Her2/neu, molecular classification.

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. The estrogen receptor has therapeutic implications [1] of utmost importance in carcinogenesis process and its inhibition through endocrine therapy – either directly by using low-estrogen agonists or indirectly, by blocking androgen hormones conversion into estrogen hormones (aromatase inhibitors) – represents the basic principle of adjuvant therapy in breast cancer [2]. Her2/neu oncoprotein is involved either in normal cell growth control or in cell division. This oncogene is associated with tumor aggressiveness [3] and with tumor cells chemo-resistance through mechanisms of gene amplification followed by a raise in transcription and protein expression level. In the patients’ management, Her2/neu status is an important predictive factor of their response to trastuzumab therapy [4].

Materials and Methods
The studied group consisted of 75 cases of invasive ductal mammary carcinomas type NOS, surgically treated within 2004–2009 in Emergency County Hospital of Craiova. The surgically specimens were fixed in 10% buffered formalin and processed by paraffin-embedded in Department of Pathology, Emergency County Hospital of Craiova. The tumors were analyzed histopathologically and scored according to “Nottingham Modification of the Scarf Bloom Richardson System”, 1992 and 1995 [5]. The method used for the immunohistochemical study 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) and Her2/neu oncoprotein (polyclonal, DAKO). The positive internal control was completed through microscopic analysis of the sections taken from diagnosis specimens. The study of estrogen (ER) and progesterone (PR) receptors immunohistochemical stain was based only on the nuclear stain for which we used a cut point of 10% positive tumor cells, without taking into consideration the intensity of reaction [6–8]. The score of Her2/neu immunohistochemical stain was determined conform to DAKO indications, scores 0 and 1+ being considered negative, score 2+ equivocal and 3+ positive [9]. The analyzed cases were distributed into four subtypes of molecular classification: luminal A (ER and/or PR+, Her2-neu-), luminal B (ER and/or PR+, Her2/neu+), Her2 (ER and PR-, Her2/neu+) and basal (ER, PR, Her2/neu-) [10, 11].

### Results

The control group consisted of 75 invasive ductal mammary carcinomas type NOS, most of them diagnosed at women aged over 50 years (76%). 49.3% of cases were classified in T2 category and 17.3% in T3, depending on the tumor size. From the point of view of differentiation degree, the most frequent tumors were low-differentiated (G3), with a percent of 53.3% of cases, followed by G2 (37.4%) and G1 (9.3%).

All the 75 cases were evaluated immunohistochemically, in terms of hormonal receptors expression, determining the tumor immunophenotype at the same time. The majority of these 75 cases, representing a percent of 73.4% were ER+, the rest of 26.6% being ER-. As far as progesterone receptor expression is concerned, 62.6% of cases were PR+ and only 37.4% were PR-. Determination of tumor immunophenotype (corroboration between hormonal receptors expression ER and PR) allowed the classification of mammary tumors, 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 analyzed 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 tumors of score 0 and score 1+ were in a percent of 81.3%. Her2/neu expression indicated a score 2+ (equivocal immunohistochemical stain) in three 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 morphochlinical 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+ and 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 tumors with negative hormonal receptors (five cases, 29.5%) and the absence of Her2/neu expression (Her2/neu score 0–1) in the tumors 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” and three 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 analyzed the correlations obtained between molecular subtypes and morphochlinical parameters and we determined that luminal A subtype characterized the groups aged over 50 years (82%), only nine cases being aged up to 50 years. The majority of cases belonging to luminal B were aged less than 60 years (83%). Out of the five cases of Her2, two cases (40%) were met at patients aged less than 50 years and three cases (60%) at the group aged 51–60 years. There was no case over the age of 60 years. The basal group was met in 90% of the cases aged over 50 years (Table 1).

### Table 1 – Histopathologic parameters of molecular subtypes

<table>
<thead>
<tr>
<th>Histopathologic parameters</th>
<th>Luminal A subtype</th>
<th>Luminal B subtype</th>
<th>Her2 subtype</th>
<th>Basal subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups [years]:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ 21–30</td>
<td>0 (0%)</td>
<td>2 (33.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>▪ 31–40</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td>1 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>▪ 41–50</td>
<td>8 (16%)</td>
<td>1 (16.6%)</td>
<td>1 (20%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>▪ 51–60</td>
<td>6 (12%)</td>
<td>2 (33.3%)</td>
<td>3 (60%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>▪ 61–70</td>
<td>17 (34%)</td>
<td>1 (16.6%)</td>
<td>0 (0%)</td>
<td>4 (36.5%)</td>
</tr>
<tr>
<td>▪ 71–80</td>
<td>14 (28%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>4 (36.5%)</td>
</tr>
<tr>
<td>▪ &gt;81</td>
<td>4 (8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>T category:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ T1</td>
<td>20 (40%)</td>
<td>1 (16.6%)</td>
<td>2 (40%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>▪ T2</td>
<td>26 (52%)</td>
<td>3 (50%)</td>
<td>1 (20%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>▪ T3</td>
<td>4 (8%)</td>
<td>2 (33.3%)</td>
<td>2 (40%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>Tumor degree:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>▪ G1</td>
<td>6 (12%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>▪ G2</td>
<td>23 (46%)</td>
<td>2 (33.4%)</td>
<td>1 (20%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>▪ G3</td>
<td>21 (42%)</td>
<td>4 (66.6%)</td>
<td>8 (80%)</td>
<td>9 (82%)</td>
</tr>
</tbody>
</table>

We investigated the relationship between molecular subtypes and tumor dimension and we observed that tumors of small dimensions (T1–T2) prevailed (92% of the cases) in luminal A, only 8% of cases in this category having dimensions categorized in T3. Regarding luminal B subtype, 83.3% of cases had dimensions classified in T2–T3 and 16.4% had small tumor dimensions specific to T1. Tumors in the subtypes with negative hormonal receptors had large dimensions.
specific to T2–T3 (60% of Her2 tumors, respectively 91% of basal subtype) (Table 1).

As concerns tumor differentiation degree, luminal A subtype was associated in percent of 58% of cases with well and moderately differentiated tumors, only 42% of the cases presented low-differentiated tumors (Figure 1).

Luminal B subtype presented moderately differentiated tumors in 33.3% of the cases and low differentiated tumors in 66.7% of the cases (Figure 2).

Her2 subtype was associated in 20% of the cases with moderately differentiated tumors G2 and in 80% of cases with low-differentiated tumors G3 (Figure 3).

Basal subtype presented a moderate and low differentiation degree in 91% of cases (Figure 4).

Figure 1 – Invasive ductal well differentiated mammary carcinoma, luminal A subtype, ×100: (A) ER+; (B) PR+; (C) Her2/neu 1+.

Figure 2 – Invasive ductal low differentiated mammary carcinoma, luminal B subtype, ×100: (A) ER+; (B) PR+; (C) Her2/neu 3+.

Figure 3 – Invasive ductal low differentiated mammary carcinoma, Her2 subtype, ×100: (A) ER-, positive internal control; (B) PR-, positive internal control; (C) Her2/neu 3+.

Figure 4 – Invasive ductal low differentiated mammary carcinoma, basal subtype, ×100: (A) ER-, positive internal control; (B) PR-, positive internal control; (C) Her2/neu -.
Discussion

The correct treatment of breast cancer is a multi-disciplinary treatment, the sequence of therapeutic methods and their aggressiveness being conditioned by the histopathologic type, tumor 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. To create a personalized therapeutic plan it is highly essential to place different categories of patients in prognosis groups based on data about tumor dimension, tumor degree, ganglionary status, lymphovascular invasion and IHC tumor profile.

The majority of patients in the control group were aged over 50 years (76%). Most tumors had large dimensions, 66.6% of the cases were categorized in T2 and T3. 53.3% of cases were low differentiated, classified in G3 category, comparatively with only 9.3% of cases of well-differentiated tumors G1.

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 that can be compared with those obtained by large studied groups and published in the specialty literature [12].

Analyzing 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 three cases (4%). In the study carried out by Rakha EA et al., these immunophenotypes had the following frequency: ER+PR+ 55.3%, ER+PR- 15.6%, ER-PR+ 3.4% and ER-PR- 27% [12].

Following the analysis of Her2/neu status of our group we realized that the cases with negative Her2/neu immunohistochemical stain (81.3%) prevailed, only 11 cases (14.8%) expressed positive Her2/neu immunohistochemical stain scored 3+. Rydén L et al. [13] shows that overexpression or Her2 gene amplification is identified in 10–30% of primary mammary carcinomas and it is correlated with an aggressive tumor 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). The subgroup characterized by the presence of immunohistochemical stain for both hormonal receptors ER+PR+ and expressing positive Her2/neu status scored 3+ had an incidence of 9.5% in our study. Our results comply with most of the studies in specialty literature and show that the presence of estrogen receptor ER and progesterone receptor PR is inversely correlated with Her2 expression [14].

Rydén L et al. [13] assert this inverse correlation between Her2 status and hormonal receptors as well and show that the percent of the tumors, which express both hormonal receptors and Her2 over-expression, is placed under 10%.

Knowing the status of hormonal receptors and Her2/neu oncoprotein 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 [10], in which the most frequent subtype is luminal A subtype, representing 71%, basal subtype was identified in 15% of cases, luminal B subtype in 8% of cases and Her2 in 6%.

We analyzed all these subtypes and we observed significant differences concerning the age, dimension and tumor degree. Thus, in our study the age groups over 50 years were characteristic to luminal A (82% of cases) and basal (90% of cases). Her2 and luminal B, characterized by positive Her2 status, were met at patients aged up to 60 years. Specialty literature asserts the correlation between luminal A and advanced ages [11] and the presence of Her2 at patients with younger ages comparatively with those belonging to luminal A [10]. The same study states that basal subtype is characteristic to younger age groups than those met in luminal A, unlike our study, which shows that the two subtypes characterize comparable age groups.

Comparing the tumors in luminal A (which do not show over-expression Her2/neu) with those characterized by positive Her2/neu status (luminal B and Her2), we determined that these are, more frequently, tumors with small dimensions and tumor degree G1–G2. Thus, out of 50 cases in luminal A, 92% had dimensions under 5 cm, T1–T2. Only four cases had tumor dimensions of over 5 cm. On the other hand, in Her2 subgroup, 60% of cases had tumor dimensions categorized in T2–T3. As concerns basal subtype, it generally presented tumors of large dimensions (91%) in T2–T3.

Upon analyzing the relationship between molecular subtypes and differentiation degree, we determined that 58% of luminal A were well and moderately differentiated. Luminal B associated tumors scored 3 in proportion of 66.7% and this percent reached 80% in the case of tumors in Her2 category. In 82% of cases, basal subtype presented tumors with low-differentiation degree G3.

The correlations made in our study are comparable with those in specialty literature. Wiechmann L et al. [10] showed that the patients in Her2 and luminal B subgroups had more frequently multifocal/multicentric tumors, tumors with larger dimensions and more lymphovascular invasions.

These authors demonstrated that the factors associated with bad prognosis (high tumor degree, large tumor dimensions, ganglionary metastasis, lymphovascular invasions, young age) are more frequently associated with tumors, which overexpressed Her2/neu. In their study, Her2 subtype was associated with higher risk of multicentric/multimodal disease and more
ganglionary metastases than luminal A subtype. Sorlie T et al. [15] and Carey LA et al. [16] showed that a follow-up survival of 10 years of these tumors is of approximately 52%, being the lowest one amongst all types of molecular tumors.

Luminal B presented an intermediary biological behavior between luminal A subtype and Her2. Basal subtype was associated with low risk of multicentric/multimodal disease and a low incidence of ganglionary metastases. Other specialty studies also assert the weak association of basal subtype with axillary metastases, comparatively with other subtypes, being associated more frequently with cerebral and pulmonary metastases [17].

Basal-like carcinoma is more frequent in cases of pre-menopause, being associated with high degree tumors with solid architecture, high mitotic index, massive inflammatory infiltration, necrosis, high risk of cerebral and pulmonary metastasis, high recurrence rate, high incidence of specific decease, irrespective of lymphnodal status and tumor size [18].

Conclusions

Immunohistochemical analysis of the studied group consisting of 75 invasive ductal mammary carcinomas of NOS type emphasized high positivity of hormonal receptors, 73.3% of the analyzed cases being ER+ and 62.6% PR+. The immunophenotype expressing both hormonal receptors ER+PR+ had the highest incidence – 58.6%, and the immunophenotype ER-PR+ had the lowest incidence – 4%. The analysis of Her2/neu status indicated that 81.3% of the cases were Her2/neu negative tumors (score 0 or 1+), 4% were equivocal (score 2+) and 14.8% were Her2/neu positive (score 3+). Her2/neu status was inversely correlated with hormonal receptors, positive Her2/neu tumors scored 3+ also being ER+PR+ in 9.5% and ER-PR- in 29.5% of the cases. We ranked the cases depending on molecular classification and we obtained the following incidences for the four subtypes: luminal A 70%, basal 14.7%, luminal B 8.3% and Her2 7%.

Her2 and luminal B subgroups, characterized by Her2/neu overexpression and variable expression for ER and PR, were met at patients aged under 60 years and had tumors of large dimensions T2–T3 (60% in Her2 cases and 83.3% in luminal B cases), low-differentiation degree G3 (66.7% for luminal B subgroup and 80% for Her2 subgroup). Luminal A subtype was associated with advanced age in 82% and was characterized by tumors of small dimensions T1–T2 in 92% of cases and with differentiation degree G1–G2 in 58% of the cases. Basal subtype, although met at patients aged over 50 years (90% of the cases), was characterized by tumors of larger dimensions (91% of the cases being T2–T3) and by a higher incidence of low-differentiated cases (82% G3).

The results of our study indicate a variability of ER and PR expression, even more emphasized when adding the evaluation of Her2/neu oncoprotein, which draws attention to breast cancer polymorphism and, consequently, to the high necessity of enforcing a personalized therapy.

References


Corresponding author
Felicia Recăreanu, PhD candidate, University of Medicine and Pharmacy of Craiova, 66 1 May Avenue, 200628 Craiova, Romania; Phone/Fax +40251–599 228, e-mail: feliciarecareanu@yahoo.com

Received: May 3rd, 2011

Accepted: September 10th, 2011