The evaluation of the immunoexpression of Her2/neu oncoprotein in ductal carcinoma in situ in association with invasive ductal carcinoma of the breast

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Abstract

The identification of breast ductal carcinoma in situ (DCIS) is a factor that increases 8–10 times the risk of developing invasive ductal breast carcinoma (DCI) later. In this study, we evaluated the immunoeexpression of the HER2/neu oncoprotein in the DCIS cases associated with DCI, both in situ and in the invasive components. We also studied the Her2/neu immunoreactivity in the cases of DCI having no DCIS association. The positive immunoreactivity (score 3) of the HER2/neu oncoprotein was present in 29 cases of high-grade DCIS having DCI associated, corresponding to the histological types comedo, solid, comedo/solid, and micropapillary. A weak-to-moderate complete membrane staining (score 2+) was determined in five high-grade DCIS and four intermediate-grade DCIS cases, belonging to the types comedo, solid, and micropapillary. The negative immunoreactivity of HER2/neu was identified in 18 cases, most of them being of low grade and belonging to the solid and cribriform types. The invasive component of the analyzed lesions indicated a HER2/neu positive reaction in 50% of lesions having DCI associated and 17.4% of the lesions having no DCIS association. The DCIS–DCI association and the DCIS histological types that were analyzed through the HER2/neu immunoexpression can stand as prognostic factors for the malignant breast lesions.

Keywords: ductal carcinoma in situ, histological types, immunohistochemistry, HER2/neu.

Introduction

The intraductal proliferative lesions are associated with different risk levels in the further development of an invasive breast carcinoma and that risk increases 8–10 times at female patients with DCIS [1].

Tavassoli FA et al. [2], including the nuclear grade, presence or absence of necrosis, as well as cytological and architectural pattern, has classified DCIS as follows: ductal carcinoma in situ of high-grade, intermediate grade, and low grade. He also described five histological types of DCIS: comedo, solid, cribriform, micropapillary/papillary, and clinging [2].

In situ lesions are heterogeneous, one third of the cases having various associated DCIS histological types [3, 4].

DCIS usually develops unilaterally, and about 22% of the cases will show DCIS or invasive breast carcinoma in the opposite breast [5].

DCIS with high grade and DCIS with low grade of malignancy seem to show different genetic changes, each of them evolving into various types of invasive breast carcinomas [5].

HER2/neu (c-erbB-2) is a transmembrane protein with 185kD as its molecular weight, produced at the level of the ErbB2 proto-oncogene, located on the long arm of chromosome 17, representing a subclass-I receptor for the corresponding growing factor [6].

The overexpression of the protein is found in DCIS, being absent at a normal breast and at the benign breast lesions [7–9].

In numerous studies, the alteration of c-erbB-2 proved to be an important event in the malignant transformation [10–13].

Some authors have reported a low HER2/neu expression in invasive ductal breast carcinomas, comparing with its expression in DCIS [8, 9, 14, 15].

The amplification of this oncogene and/or the overexpression of the HER2/neu oncoprotein were observed in approximately 30% of the DCIS, most of which are of high grade (60–80%) [16, 17].

In this study, we evaluated the immunoeexpression of the HER2/neu oncprotein in the DCIS cases associated with DCI, both in situ and in the invasive components. We also studied the Her2/neu immunoreactivity in the cases of DCI having no DCIS association.

Materials and Methods

The study included 127 cases of invasive ductal breast carcinoma diagnosed in 2009 in the Pathology Laboratory of Emergency County Hospital of Craiova.
Lesions were classified as proposed by Tavassoli FA et al., classification accepted and used by the World Health Organization – 2003, following the nuclear grade, presence or absence of necrosis, as well as the architectural and cytological pattern [2].

For the immunohistochemical analysis, serial sections of 3-4 μm thick from the paraffin blocks were made, which were applied to poly-L-lysine-treated slides, and then dried for one hour on the thermostat at 56°C.

A rabbit polyclonal anti-human HER2/neu antibody was used (Dako, Redox, Romania) with a dilution used of 1:250. The detection and visualization system used was Envision kit (Dako, Redox, Romania).

For the interpretation of the HER2/neu immunoreactivity, the ASCO/CAP (American Society of Clinical Oncology/College of American Pathologists) grading system was used, for both carcinoma in situ (DCIS) lesions and invasive ductal breast carcinoma lesions [18] (Table 1).

<table>
<thead>
<tr>
<th>Score 0</th>
<th>No staining or staining of the cell membrane is observed in less than 10% of the tumor cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score +</td>
<td>A fade, slightly perceptible staining, or a discontinuous staining of the cell membrane is observed in more than 10% of the tumor cells. The cells are stained only on their membrane.</td>
</tr>
<tr>
<td>Score 2+</td>
<td>A weak to moderate complete staining of the cell membrane is observed in more than 10% of the tumor cells.</td>
</tr>
<tr>
<td>Score 3+</td>
<td>A strong complete staining of the cell membrane is observed in more than 30% of the tumor cells.</td>
</tr>
</tbody>
</table>

The scores 0/1+ were interpreted as negative immunoreactivity, the score 2+ as weak positive immunoreactivity, and the score 3+ as positive immunoreactivity.

Results

Among the 127 cases of invasive ductal breast carcinoma that were examined, breast ductal carcinoma in situ was associated in 58 cases (45.66%).

Most of the cases diagnosed with invasive ductal breast carcinoma belong to the seventh decade of life (29 cases), and for cases having DCIS associated, most of them were in the sixth decade of life (24 cases).

All patients were females, from the age of 32 years as the youngest to 91 years as the oldest.

The histopathological analysis of the cases with invasive tumors having DCIS associated showed the presence of the types: comedo in 26 cases (44.82%), solid in 16 cases (27.58%), cribriform in seven cases (12.06%), papillary in two cases (3.44%), and micro-papillary in one case (1.72%). We observed associations between the in situ lesions in six cases (10.34%) (Table 2).

<table>
<thead>
<tr>
<th>DCIS grade</th>
<th>Histological types of DCIS / No. of cases</th>
<th>Her2/neu expression – No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>High grade</td>
<td>Comedo / 26</td>
<td>3+ 2+ 0/1+</td>
</tr>
<tr>
<td>– G3</td>
<td>Solid / 4</td>
<td>- - -</td>
</tr>
<tr>
<td></td>
<td>Comedo + Solid / 5</td>
<td>3 2 -</td>
</tr>
<tr>
<td></td>
<td>Micropapillary / 1</td>
<td>1 - -</td>
</tr>
<tr>
<td>Intermediate grade – G2</td>
<td>Solid / 2</td>
<td>- 2 -</td>
</tr>
<tr>
<td></td>
<td>Cribriform / 2</td>
<td>- 2 -</td>
</tr>
<tr>
<td>Low grade</td>
<td>Comedo / 26</td>
<td>3+ 2+ 0/1+</td>
</tr>
<tr>
<td>– G1</td>
<td>Solid / 10</td>
<td>- - 10</td>
</tr>
<tr>
<td></td>
<td>Cribriform / 5</td>
<td>- - 5</td>
</tr>
<tr>
<td></td>
<td>Papillary / 2</td>
<td>- - 2</td>
</tr>
<tr>
<td></td>
<td>Micropapillary + Cribriform / 1</td>
<td>- - 1</td>
</tr>
</tbody>
</table>

The malignancy grades of the investigated DCIS cases were high in 38 cases (62%), low in 18 cases (31%) and intermediate in four cases (7%) (Table 2).

Over the high-grade DCIS cases, the comedo type was predominant, over the low-grade cases, the solid type was predominant, and over the intermediate-grade cases, the solid and cribriform types were equally predominant.

The analysis of HER2/neu immunoreactivity in breast ductal carcinoma in situ showed different scores, depending on the histological types (Table 2).

The positive immunoreactivity of the HER2/neu protein (score 3+) was identified in 29 cases of high-grade DCIS, corresponding to the histological types as follows: comedo (21 cases), solid (four cases), comedo/solid (three cases), and micropapillary (one case) (Figures 1 and 2).

At these cases, we noted an intense and continuous staining of the cell membrane in over 30% of the tumor cells.

A weak to moderate complete staining of the cell membrane for Her2/neu (score 2+), was established in five cases of high-grade DCIS, corresponding to the types comedo (three cases) and comedo/solid (two cases) (Figure 3b).

Also, the weak positive immunoreaction was present in all the four cases of intermediate-grade DCIS (Figure 3, c and d).

The cases with a weak positive immunoreaction of the Her2/neu oncoprotein showed a weak to moderate continuous staining of the cell membrane in over 10% of the tumor cells.

The negative immunoreactivity of HER2/neu (score 0/1+) was present predominantly in the low-grade in situ lesions, with the histological types as follows: solid (10 cases), cribriform (five cases), papillary (two cases) and micropapillary/cribriform (one case) (Figure 4b).
Two cases of breast ductal carcinoma *in situ* of high grade (of comedo histological type) indicated a negative immunoreactivity of HER2/neu. At these cases, the staining of the cell membrane was either absent or discontinuous, barely perceptible, for more than 10% of the tumor cells.

The invasive component of the analyzed DCIS cases revealed a HER2/neu positive immunoreactivity in 29 cases, weak positive in 19 cases, and negative in 10 cases (Table 3).

The 69 cases of DCI not associated with DCIS showed a HER2/neu positive immunoreactivity in 12 cases, weak positive in 11 cases, and negative in 46 cases (Table 3).

<table>
<thead>
<tr>
<th>HER2/neu expression</th>
<th>No. of cases of DCI associated with DCIS</th>
<th>No. of cases of DCI having no association with DCIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 3+</td>
<td>29 (50%)</td>
<td>12 (17.4%)</td>
</tr>
<tr>
<td>Score 2+</td>
<td>19 (32.8%)</td>
<td>11 (16%)</td>
</tr>
<tr>
<td>Score 0/1+</td>
<td>10 (17.2%)</td>
<td>46 (66.6%)</td>
</tr>
</tbody>
</table>
Figure 3 – Her2/neu stain, score 2+: (a) Comedo DCIS associated with DCI (ob. 10×); (b) Comedo DCIS associated with DCI (ob. 20×); (c) Solid clear-cell DCIS associated with DCI (ob. 10×); (d) Solid clear-cell DCIS associated with DCI (ob. 20×).

Figure 4 – Her2/neu stain, score 1+: (a) Papillary DCIS associated with DCI (ob. 4×); (b) Papillary DCIS associated with DCI (ob. 20×).

Discussion

In our study, the DCIS associated with DCI was present in 45.66% of the analyzed cases and was predominant in the sixth decade of life (24 cases), which complies with the data throughout the literature. In a survey made in 2005 on the population of Australia, 51% of the DCIS cases were diagnosed at patients aged from 50 to 69 years [19]. After SEER Cancer Statistics, DCIS is predominant over the age interval of 60–79 years [20]. Page DL and Dupont WD argue that patients aged over 60 years at the moment of diagnosis of DCIS, have a 40% absolute risk of developing DCI in the next 20 years [21].

In this study, the in situ lesions were heterogeneous, presenting associations between the histological types in 10.34% of the cases. Other studies have identified the presence of the association between the histological types at the in situ lesions in about one third of the examined cases [3, 4].

The over-expression (score 3+) of the HER2/neu protein was present in 50% of the DCIS lesions; corresponding to the high histological grade (histological types of comedo, solid, comedo+solid, and micro-
Similar immunohistochemical studies indicated the membrane immunoreactivity of the HER2/neu oncoprotein in 42–61% of the DCIS cases [9, 22–25].

Inaji H et al. noted the overexpression of HER2/neu being significantly higher at the comedo type than at cribriform and micropapillary types [26]. The HER2/neu overexpression was identified in 8–100% of the cases of in situ comedo type associated with nuclear pleomorphism [22, 25, 27].

Similar studies have reported that the DCIS HER2/neu expression is present in over 40% of the cases, predominantly in patients with comedo histological type/high-grade DCIS rather than in patients with non-comedo/low-grade DCIS [28]. Other studies have reported the over-expression of HER2/neu oncoprotein in cribriform and micropapillary DCIS [22, 25, 30].

Another study, which analyzed the expression of HER2/neu in DCIS pure lesions and the DCI lesions of HER2/neu was found in 40–60% of the evaluated DCIS cases, predominantly at the large-cell comedo type [3, 25, 29].

The HER2/neu scores 2+ and 0/1+ were present in 32.8% and respectively 17.2% of the analyzed DCIS cases. Most of the DCIS cases without HER2/neu expression belonged to the histological types of solid (G1, 10 cases) and cribriform (G1, five cases). Most reports mention the absence of the over-expression of HER2/neu oncoprotein in cribriform and micropapillary DCIS [22, 25, 30].

The invasive component present in association or no association with the in situ component, had a HER2/neu positive immunoreaction (score 3+) in 50% and respectively 17.4% of the cases. A few studies have suggested that HER2/neu protein over-expression is more frequent in the in situ component of the breast carcinomas than the invasive component [7, 31, 32]. A high expression of HER2/neu was found in 40–60% of the evaluated DCIS cases and in 20–30% of the cases having DCI [33, 34].

Further study, which analyzed the expression of HER2/neu in the DCIS pure lesions and the DCIs lesions with in situ component, reported the HER2/neu over-expression in 34% and respectively 56% of the cases, the invasive component being positive in 58% of the analyzed cases [35]. Alfred DC et al. reported a lower incidence of the HER2/neu expression at the cases DC1 associated with DCIS, than at the cases having no such association [7]. These results can be explained by the fact that HER2/neu expression is low in situ lesions that evolve to invasive lesions or that a subset of invasive carcinoma may develop de novo through mechanisms that are independent of HER2/neu.

Conclusions

The immunohistochemical analysis of the HER2/neu expression indicated a positive immunoreactivity in 50% of the cases of high-grade DCIS associated with DCI, the carcinoma in situ comedo and solid histological types proving to be the most aggressive. In the absence of the association DCIS with DCI, the invasive lesion had a HER2/neu positive immunoreaction at a reduced number of cases (17.4%).

The DCIS–DCI association and the DCIS histological types that were analyzed through the HER2/neu immunoexpression, can stand as prognostic factors for the malignant breast lesions.

References


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