HER2 Expression Discordance between Ductal Carcinoma *In situ* and Invasive Breast Carcinoma. How to Analyze Oncotype DX

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Abstract: Although histologically invasive breast carcinomas can be of no special subtype (ductal) or of special subtypes (as lobular carcinoma), based on their immunohistochemical and molecular features, they are subclassified in four different groups: luminal A, luminal B (with and without HER2 overexpression), HER2 subtype and triple negative. They vary in their gene expression signature, biological potential and clinical course. Luminal A subtype is considered to have a better prognosis and most are treated with hormone therapy alone after surgery because they express hormone receptors and show a low proliferation index. Oncotype DX (ODX) is a molecular score assay that estimates recurrence risk for early-stage hormone receptor-positive, human epidermal growth factor receptor 2 (HER2) negative breast cancer. It can predict which tumours may benefit from adjuvant chemotherapy. It has been reported that occasionally a breast carcinoma can have immunohistochemical and molecular differences between the *in situ* and the invasive components. We report one case that may lead us to misinterpret ODX results, where the *in situ* ductal component amplified HER2 gene while invasive component did not. Therefore, carefulness must be taken when evaluating ODX results, and be sure that we are evaluating the invasive component.

Keywords: HER2, DCIS, FISH, ODX.

INTRODUCTION

Breast cancer is the most frequent carcinoma in women. It is the fifth cause of death from cancer overall, but it is still the most common cause of cancer death in women in both developing and developed countries [1].

Gene expression studies have shown that there is a considerable diversity among breast carcinoma, both biologically and clinically [2]. St. Gallen classification divides breast cancer into 4 subgroups that vary in their gene expression signature, biological potential and clinical course. The classification includes: luminal A, luminal B (with and without HER2 overexpression), HER2 subtype and triple negative [3].

Ductal carcinoma *in situ* (DCIS) and invasive components of breast carcinoma usually show the same immunophenotype in both components of the same tumour. However, up to 1% of cases show different immunohistochemical profiles among these components [4, 5].

ODX (Genomic Health, Redwood City, CA, EE. UU) is a 21-gene breast cancer score assay that estimates recurrence risk for early-stage hormone receptor-positive, HER2-negative breast cancer [6]. It supplies additional prognostic information and can be predictive of benefit from adjuvant chemotherapy [7]. The ODX test is currently used worldwide for routine evaluations in conjunction with Guidelines from the American Society of Clinical Oncology (ASCO), the National Comprehensive Cancer Network (NCCN), and others [8, 9].

We report a case where the invasive component shows luminal A immunophenotype and DCIS component shows HER2 immunophenotype. We discuss the interpretation of ODX results in the surgical specimen and in the core needle biopsy.

CASE REPORT

A screening mammogram performed on an asymptomatic 49-year-old woman demonstrated a BI-RADS 2 lesion in the left breast. Gynaecological-obstetric history showed early menopause at 39 years and hormone replacement therapy for 5 years. On examination, she had small breasts with increased density in the upper outer quadrant of the left breast of 4 cm in length and an ipsilateral rolling adenopathy.

A core needle biopsy was performed and four cylindrical fragments were evaluated. Histological study demonstrated an invasive breast carcinoma of no special subtype (grade I of Elston-Ellis), without associated DCIS component (Figure **1A**). Invasive carcinoma cells were positive for estrogens (Allred score 8) and CK19, while negative for HER2 protein

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(Figure **1B**) and progesterone receptors with a low proliferation index (1%, studied with Ki67). Therefore, it was classified as luminal A subtype.

One month after diagnosis a left mastectomy was performed with sentinel node biopsy. Macroscopic study demonstrated a mastectomy specimen that measured 12.5x10x3.5cm. Serial sections showed two indurated lesions separated by more than 2 cm that were located in upper-outer quadrant and in outer inter quadrants measuring 2x1cm and 1x1cm respectively. We studied 2 left axillary sentinel lymph nodes that were negative for carcinoma metastasis.

In the microscopic study, upper outer quadrant lesion was diagnosed as an invasive breast carcinoma of no special type with a peripheral rim of associated ductal carcinoma *in situ* component (Figure **1C**). The whole tumour measured 1.8cm, while the central invasive component measured 1cm. The second tumour was a ductal carcinoma *in situ* grade II without necrosis and measured 1x1cm.

ODX was requested on the surgical specimen and 15 unstained sections were evaluated. The study determined that there was amplification of the HER2 gene and recurrence score: 38, in the submitted samples. These results were disharmonious with core needle biopsy diagnosis.

Surgical specimen tissue sections were reviewed and we observed that the invasive component had

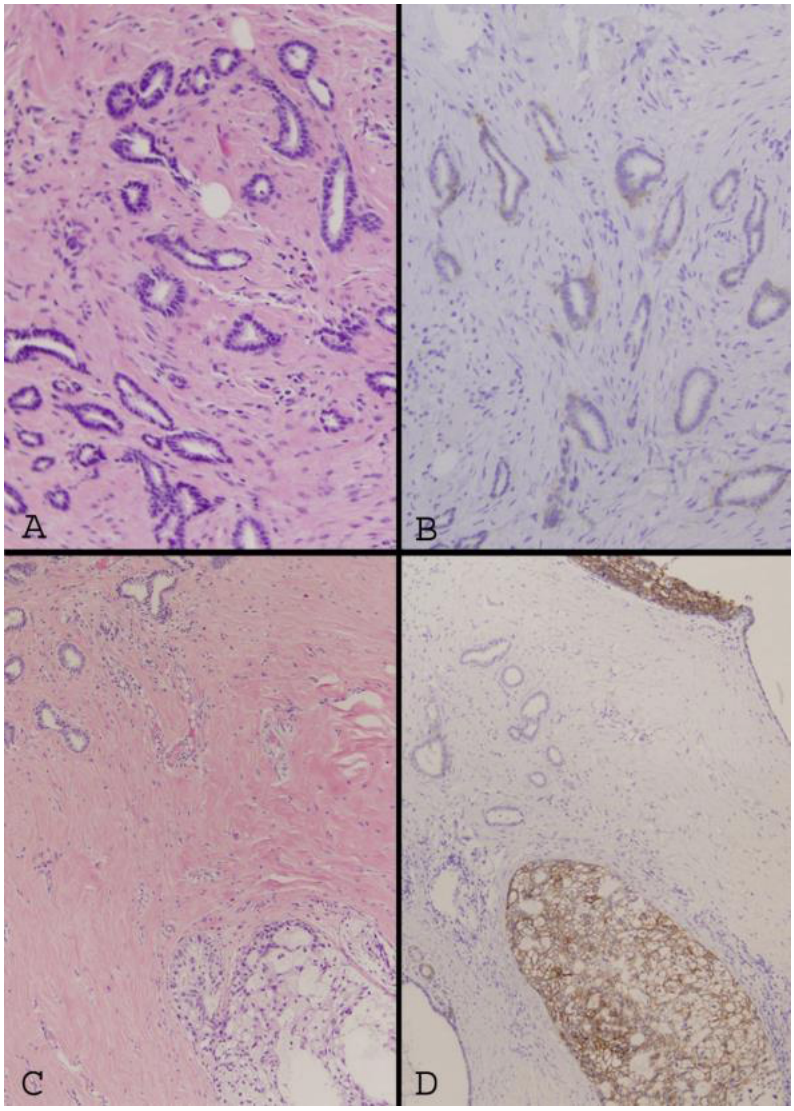

Figure 1: A. Invasive carcinoma in needle core biopsy (H & E stain, x200 aprox). **B**. Invasive carcinoma showing HER2 IHC negative staining (Herceptest IHC, x200 aprox). **C**. *In situ* and invasive component of breast carcinoma (H&E stain, x100 aprox). **D**. *In situ* and invasive carcinoma showing discordant Her2/neu protein expression. 3+ IHC membrane staining is noted within the DCIS and negative staining within the invasive component (Herceptest ICH, x100 aprox).

practically disappeared as a result of serial cuts, so DCIS component predominated and it was the part of the lesion that overexpressed HER2 (Figure **1D**) and amplified HER2 gene by fluorescence *in situ* hybridization (FISH) tests (HER/cep17 ratio= 3,5 / average HER2 copy number signals/cells= 7), unlike the minimum invasive component that did not overexpressed HER2 protein (Figure **2A**).

Invasive component was better represented on core needle biopsy, so we repeated immunohistochemical and Fluorescence *in situ* hybridization (FISH) tests for HER2 protein over these sections. We obtained negative results (HER/cep17 ratio= 1,5 / average HER copy number signals/cells= 1,9) (Figure **2B**), so we performed ODX over the core needle biopsy. The study determined that there was not amplification of the HER2 gene and recurrence score: 15.

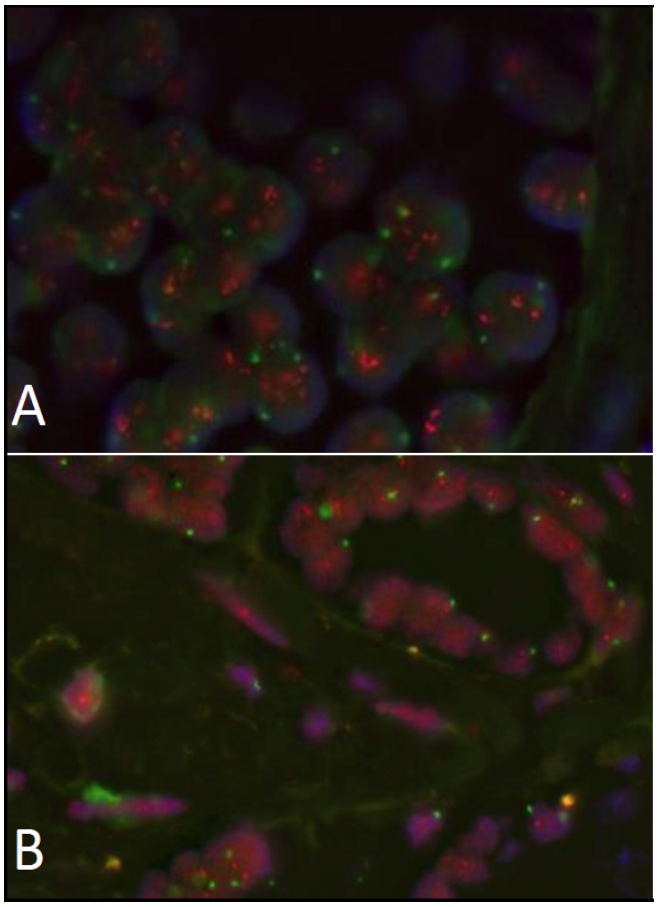

Figure 2: A. *In situ* component showing high HER2/neu amplification by FISH. **B**. Invasive ductal carcinoma showing no HER2/neu amplification.

As the infiltrating component is the one that determines prognostic course, it was concluded that it was a Luminal A carcinoma with a low recurrence rate, without benefits from adjuvant chemotherapy.

To perform the immunohistochemical staining and molecular studies, 4 mμ tissue sections of the paraffin embedded material were used on slides treated with poly-L-lysine using antibodies to the following antigens: ER, PR, CK19, and Ki67 (Agilent Technologies, Inc. Santa Clara USA). Dako-OMNYS equipment was used for immunostaining. The immunodetection was carried out using the ENVISION FLEX, HIGH pH visualization system (Agilent Technologies, Inc. Santa Clara USA).

Hormone receptor evaluation was made according to ASCO/CUP 2010 recommendations [10].

Proliferation index evaluation (Ki67) was performed according to recommendations from the international Ki-67 in Breast Cancer Working group 2011 [11].

To evaluate HER2 overexpression IHC techniques were performed according to the manufacturer of the HercepTest kit (Agilent Technologies, Inc. Santa Clara USA). HER2 gene amplification was determined by FISH using a dual-colour probe, Food and Drug Administration-approved PathVysionTM HER2/neu DNA Probe Kit and Paraffin Pre-treatment Kit (Agilent Technologies, Inc. Santa Clara USA) [10].

HER2 protein overexpression evaluation (IHQ) and HER2 gene amplification (FISH), were performed according to ASCO / CUP 2013 recommendations [12].

DISCUSSION

Breast cancer is a heterogeneous disease. Most studies consider the *in situ* component associated with invasive carcinomas as a precursor lesion for cancer. A correlation between the molecular changes involved in the progression of the *in situ* component to the invasive component has been found. If left untreated, many of these *in situ* lesions will progress to invasive cancer. However, there are not well established histological, immunohistochemical or molecular criteria that allow us to discern which of these *in situ* lesions will progress to invasive carcinomas [3].

HER2 overexpression DCIS rises up to 50% of the cases, which has led to the hypothesis that HER2 amplification may be an early genetic change in the carcinogenesis of breast cancer. Local recurrence of DCIS varies depending on the studies (16-60%) [13]. Despite its prognostic value, clinical implications of DCIS HER2 overexpression have not yet been clarified; being HER2 overexpression more frequent in high-grade DCIS lesions (50%) than in invasive cancer samples (20-30%) [14].

Invasive carcinomas may have a variable *in situ* associated component [4]. Although immunohistochemical features are usually similar in both components there may be differences in 1% of cases. There are several reports that have described cases of invasive breast carcinoma that, unlike their accompanying *in situ* component, do not amplify for HER2 [5]. Therefore, there are cases, such as ours, in which HER2 status is different. Some studies such as Park *et al*. [4] showed four cases where *in situ* ductal component amplified HER2 while the accompanying invasive component did not. In this same study, a higher prevalence HER2 overexpression was identified in pure DCIS than in those with micro invasive or invasive carcinoma. These data are very important to correctly evaluate ODX results. In our case, we did not studied whether there was sufficient amount of invasive carcinoma being represented in the unstained sections of the surgical specimen submitted.

After receiving ODX report, whose results determined that there was HER2 gene amplification, new cuts of the tumour were made. In these new sections, it was observed that there was a predominance of DCIS component that overexpressed HER2, compared to the invasive component that was poorly represented (infiltrative component did not overexpressed HER2). We consider that HER2 overexpression in the *in situ* component was responsible for ODX assay results.

Invasive carcinoma is the lesion with prognostic value. Therefore, we decided to send new core needle biopsy tissue sections to carry out the ODX test. In the new sections invasive component was represented without the presence of a ductal *in situ* component. The results of this study determined that it was a low recurrence risk tumour, without HER2 amplification, and adjuvant chemotherapy was unnecessary.

CONCLUSIONS

As previous studies have shown, and our study shows, in some cases, DCIS and invasive carcinoma do not have the same immunohistochemical or molecular profile [4, 5]. This variability can lead us to make mistakes when interpreting FISH and ODX results. Immunohistochemistry and HER2 must be performed in the surgical specimen and if they do not have the same immunohistochemical features and HER2 status it is essential to ensure that the component we analyse by ODX is the invasive and no the DCIS component, because the former is the one taken into account for therapy decisions and patients prognosis, so microdissection may be performed if necessary.

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