

# HER-2 NEU IN BREAST CANCER PATIENTS. IMMUNOHISTOCHEMICAL COMPARISON WITH MONO AND POLYCLONAL ANTIBODIES. AMPLIFICATION WITH CHROMOGENIC IN SITU HYBRIDIZATION (CISH)

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## ABSTRACT

Breast cancer is an oncological problem, that represents a rate of 20.4 per 100 000 habitants (1). The oncogen Her-2/Neu is a molecular marker used in prognosis and treatment of this disease. **Aim:** To determine by means of immunohistochemistry (IHC) with polyclonal (Herceptest) and monoclonal (M.Ab) antibodies, the over expression of Her2 protein, in 2+ and 3+ patients using Chromogenic in Situ Hybridization (CISH). **Materials and Methods:** 246 patients diagnosed with breast cancer were evaluated with IHC and CISH. **Results:** 96.3% of the patients were diagnosed as infiltrating ductal carcinoma, NTH II (80.9%). Prevalence age was 40 to 49 years old (27.8%). Expressed the protein 67 patients (27.2%) with (Herceptest); 70 patients (28.5%) with M.Ab. Using the CISH technique it was found that the 2+ group with M.Ab. amplified in 69% of the cases; with Herceptest in 78.38% of the cases and 3+ group amplified Her-2 protein in 100% of the cases with both antibodies, performed by CISH.

**Keywords:** Breast cancer, Her-2/neu, CISH.

## INTRODUCTION

Breast cancer is the most frequent malignant neoplasia in women. WHO reports that breast cancer accounts for 22% of the different cancer types in women (2). There are both prognostic and predictive indicators for this malignancy; one of the molecular markers is oncogen Her-2; which codifies a 185 KDa glycoprotein, located in the chromosomic region 17q12-21 (3). The amplification of oncogen Her-2 is presented in 20 to 30% of breast cancers and results in an increase of the protein expression. Slamon *et al*; (7) observed the significant relation between the amplification of oncogen Her-2 and the poor prognosis in breast cancer patients. The oncogen Her-2 over expression identification has been demonstrated using several techniques, such as Southern Blot, Spot Blot, Polymerase chain Reaction (PCR), Immunehistochemistry (IHC), Fluorescent *In Situ* Hybridization (FISH); and the *Chromogenic In situ Hybridization* (CISH), with a recent set up. Both FISH and CISH have the advantage to analyze morphology and amplification simultaneously. These features do not suffer changes with dilution artifacts, which affect other methods to identify genetic amplifications. These methods can be performed in tissues fixed with buffered formaldehyde and blocked in paraffin (4). These methods are more sensitive than DNA amplification methods, with a sensitivity of 98% and specificity of 100%. The amplification of oncogen Her-2 is present in the prediction, evolution and prognosis of the breast cancer malignancy.

## MATERIALS AND METHODS

Were included in this study 246 breast cancer patients. The IHC panel was performed to all the cases. This methodology included the over expression of Her-2 protein for 2+ and 3+ cases. The gen amplification was developed by CISH.

To determine the Her-2 protein over expression by means of IHC, monoclonal and polyclonal antibodies kits were used. The monoclonal antibody used was CB-11 (Cell Marque). The polyclonal antibody kit used was Herceptest (Dako Ref. K5204), chosen by its high affinity to HER-2 in routine material, formaldehyde fixed and paraffin blocked. Results were interpreted using Herceptest manual (Dako Cytomation).

The amplification of Her-2 gen Zymed Spot-Light (Ref. 84-0146) was determined using chromogenic in situ hybridization (CISH). This technique was performed in tissues fixed in formaldehyde and blocked in paraffin, using the following procedure: pre-treatment (tissue cuts), denaturalization and hybridization; washing, counter staining, microscopic observation in luminous field. The CISH results interpretation was performed according to Zymed Spot-Light Manual.

## RESULTS

From the population studied, 237 patients presented infiltrating ductal carcinoma (96.3%) while nine patients presented infiltrating lobular carcinoma (3.7%). Prevalence age was 40 to 49 years old in 27.8% of the cases; 70 patients (28.5%) showed the over expression of Her-2 gen by means of monoclonal antibodies (see Table 1); 67 cases (27.2%) displayed the gen over expression with polyclonal antibodies (Table 2). Her-2 gene amplification with CISH in 2+ over expressed patients (M.Ab) was in 69.23%, and P.Ab in 73% of the cases. The patients group with a 3+ over expression amplified the gene in 100% of the cases with both antibodies (see Table 3, 4 and Fig. 1-2)

**Table 1. Over expression M.Ab**

M.Ab	Frequency	Percentage
Negative	176	71,5
Positive	70	28,5
Total	246	100,0

**Table 2. Over expression Herceptest**

Herceptest	Frequency	Percentage
Negative	179	72,8
Positive	67	27,2
Total	246	100,0

### Amplification CISH 2+ and 3+ Herceptest    Amplification CISH 2+ and 3+ M.Ab

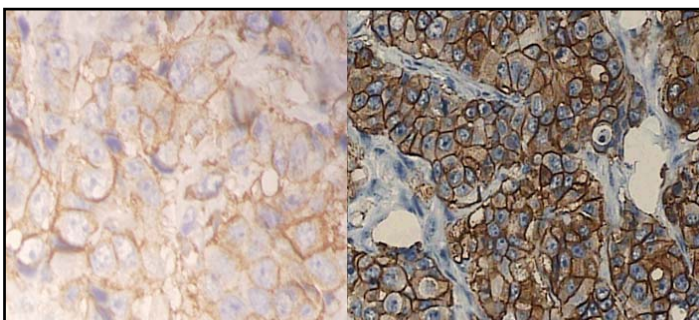
Table 3.

P. Ac.	CISH				Total	%
	Negative	%	Positive	%		
++	8	21.62	29	78.38	37	100
+++	0	0.00	30	100	30	100
Total	8	11.94	59	88.06	67	100

Table 4.

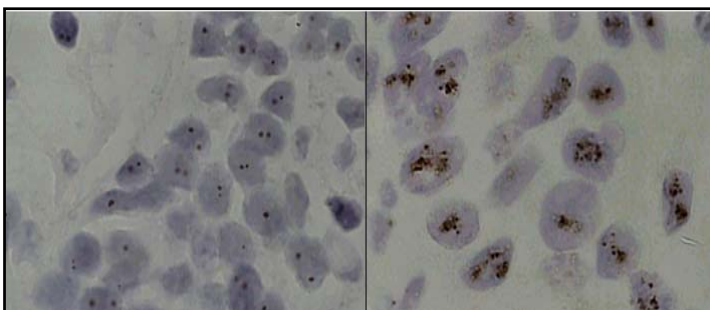
M.Ac.	CISH				Total	%
	Negative	%	Positive	%		
++	12	30.77	27	69.23	39	100
+++	0	0.00	31	100	31	100
Total	12	17.14	58	82.86	70	100

## Over expression of Her2 protein



**Figure 1.** Score 2+                      Score 3+

## Amplification of Her-2 with CISH



**Figure 2.** Without amplification    High amplification A

## CONCLUSIONS

The aim of the present work was the comparison of two methods to amplify Her-2/neu gen, with mono and polyclonal antibodies, and the relation of this features with the Chromogenic *in Situ* Hybridization (CISH). Several studies with other techniques led us to establish this methodology, through the comparison and the possible relationship with an alternative *in situ* hybridization.

The analysis reveals the Her-2/neu over expression with polyclonal antibodies (A-0485) in 27.2% of the cases. The amplification with monoclonal antibodies (CB-11) happened in 28.5% of the cases. Patients graded 2+ amplified via M. Ab in 69.23% of the cases, 78.38% of the cases amplified with Herceptest. All the patients, graded 3+ amplified with both antibodies. This is a statistically significant finding. Bhargava *et al.*, (9) used CISH to report the amplification in 3+ cases. In this work 89% of the 3+ cases amplified Her-2 gen.

Histological grading by itself is not accepted as a prognosis factor. However, several studies proved that 3+ histological graded is associated with IHC markers, related to poor prognosis (5). In our study group, 96.3% of the patients showed infiltrating ductal carcinoma, and 3.7% with lobular infiltrating carcinoma. Amplified the gene the 73.68% of ductal carcinoma; meanwhile lobular carcinoma did not amplified Her-2/neu. Simpson *et al.*,(6) demonstrated that patients with comprised axillaries nodules and mitotic proliferative activity will have a low survival rate. This finding has been reported in other

investigations (6). From our patients group, women with negative lymphatic ganglia amplified Her-2 gen in 50% of the cases. Patients with metastatic lymphatic ganglia amplified the gen in 100% of the cases.

Up to date there is no full agreement regarding the use of the presented techniques. The more appropriate method to use as a test to identify the amplification of the oncogen Her-2 is still discussed (7). Nowadays more sensitive techniques are used, such as Chromogenic *in situ* hybridization (8). This method uses tissue blocked in paraffin, allowing evaluate several samples at the same time in identical conditions. The interpretative capacity is improved and the cost of the analysis decreases if is compared with FISH. Nevertheless, this technique is not approved by FDA at the moment.

Our experience with IHC techniques for mono and polyclonal antibodies has been positive in the evaluation and the assessment of the over expression of Her-2 protein. CISH is an accurate technique, with less technical and economical demands if it is compared to FISH. CISH must be recommended as a validation test of genetic expression in 2+ cases.

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