Research Article

IMMUNOHISTOCHEMISTRY WITH MONOCLONAL ANTIBODIES HER2/NEU PREDICTS SURVIVAL IN BREAST CANCER NATIVE PERUVIAN PATIENTS

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Abbreviations: HER2/neu, human epidermal growth receptor factor 2/neuro glioblastoma derived oncogene homolog; **ELISA**, enzyme-linked immuno assay; **FISH**, Fluorescent In Situ Hybridization; **OS**, overall survival; **TNM,** tumor, nódule, metastasis.

Abstract

Breast cancer is a heterogeneous pathology. There are subgroups with different molecular characteristics, and they must be identified to direct the therapy and to have a clear prognosis. HER2/neu (human epidermal growth receptor factor 2/neuro glioblastoma derived oncogene homolog) receptor overexpression is a characteristic that must be evaluated in each population. We apply the monoclonal antibody technique in the indigenous population of Peru, we also correlate the presence of the receptor with global survival. It's an experimental, prospective and analytical study. Twenty three samples were evaluated, obtained from patients with adenocarcinoma of the breast. The HER2/neu expression was determined by immunohistochemical technique (monoclonal antibodies). The results were verified 10 cases were positive for overexpression (43.47%). The negative cases were 13 (56.52%). The overall survival at 3 years was 69.90% for the Her2/neu positive and 85.00% for negative, showed statistically significant differences (p=0.017). Finally, it was feasible to apply the monoclonal antibody technique and to statistically correlate HER2/neu overexpression with TNM clinical stage, predicting less survival when present. Applying an immunohistochemical technique is feasible in native Peruvian women. The main benefit is to apply biological therapy with monoclonal antibodies, according to their molecular profile.

1. Introduction

Breast cancer is the second leading cause of cancer in Peru, after exocervix cancer. In the Hispanic population of the United States, it ranks first in incidence, with 19,800 cases [1]. In recent years, it is evaluated to know the correlation of molecular variables with survival. This will define groups with a greater probability of therapeutic Benefit [2].

The clinical classification of breast cancer is based on the presence of receptors (proteins). Among these we have estrogen, progesterone and HER2/neu. In recent years, the study of these proteins is considered canonical for cancer patients. In some regions of Latin America, they cannot be done for lack of technology. It is relevant to consider extending its use, through the monoclonal antibody technique, for the therapeutic benefit of the patient.

Immunohistochemistry is a histopathological study based on the molecular formation of antigen-antibody [3]. This method determines the subtypes of breast cancer, as part of their genetic expression. Her2/neu receptor (human epidermal growth receptor factor 2/neuro glioblastoma derived oncogene homolog receptor) amplification has been studied using different analytical methods, in addition to immunohistochemistry, such as the Southern, Western-blot, ELISA (enzyme-linked immuno assay) and FISH (Fluorescent In Situ Hybridization) methods.

This trial seeks to determine the overexpression of Her2/neu, also called as ERBB2 protein human, in breast adenocarcinoma and its correlation with OS (overall survival) [4]. The advantage of expanding this theme is to provide a technological base for the therapeutic use of monoclonal antibodies in native populations. The disadvantage over other techniques is the high cost, but the clinical benefit for the patient always justifies its application.

In the early 1980s, monoclonal antibodies that reacted against cell membrane antigens were studied. Her2/neu (ERBB2 protein human) is a transmembrane tyrosine kinase receptor, encoded by the proto-oncogene with the same name. When the receptor is heterodimerized with HER1, HER3 or HER4, it activates a chain of signals that lead to cell proliferation and survival [5]. Likewise, Her2 can undergo genetic mutation or overexpression, which leads to cellular transformation. This event is recognized in many malignancies, such as breast cancer. Therefore, their study is useful for cancer patients.

Countries like Peru can only benefit their patients with monoclonal antibody technology, while conducting research in their native population (american indians). The objective of applying the immunohistochemical technique in breast cancer, seeks to benefit and improve survival, according to the molecular profile of the targeted treatment.

2. Material and Methods

2.1. Cases-studied

Twenty three patients diagnosed with primary breast cancer were evaluated (Peru's native population). We analyzed the expression of HER2/neu by immunohistochemistry, from November 2016 to June 2017. Subsequently the patients had a prospective follow-up of 3 years (36 months).

The inclusion criteria were: pathological diagnosis of adenocarcinoma of the breast, biological samples with enough tissue (>5 mm) to perform representative cuts. Having a complete medical history was also an inclusion criterion. The exclusion criteria was not having a FISH test result. This requirement occurred in patients with Her2/neu 2 (+), since it is a state that requires confirmation to be considered with overexpression.

2.2. Immunohistochemistry

The evaluation of HER2/neu was performed according to the recommendations defined and updated by the American Society of Medical Oncology [6]. Thirty biological samples, corresponding to the same number of patients, were evaluated. Seven samples were excluded (six due to an incomplete immunohistochemical report and one due to not having a clinical history on file).

Paraffin sections were cut and mounted on slides. Subsequently were melted at 65°C and then dipped into xylene to remove the paraffin. Rehydrating tissues, slides were washed with distilled water and dipped into a aqueous solution of 3% peroxide for 3 min and rinsed with Tris buffer. Heat retrieval was done with citrate buffer for 40 min at 95°C. It was then brought to room temperature, placing the slides in Tris-Saline buffer. The primary mouse antibody was added to the tissue section, for 1 hour. Subsequently, the tissue was washed with Tris buffer. The tissue was incubated with mouse biotinylated antibody for 10 minutes (Dako). After rinsing with Tris buffer, a solution of chromogen, 3, 3'-diaminobenzidine, Tris buffer and H2O2 (0.016%) was added. The slides were subsequently washed in distilled wáter.

2.3. Ethical Aspect

The study protocol was reviewed and approved by the National University of San Agustín. Informed consent was obtained from all included patients.

2.4. Statistics Analyses

Validation of technical results required the Kappa index. Fisher's exact test was used to evaluate the categorical variables. Overall survival was calculated using the Kaplan-Meier method and compared with the Logrank test. All data was analyzed using IBM® SPSS® version 16.

3. Results

3.1. Clinical Findings

Our results are compared in Table 1, considering two indicators: TNM (tumor, nódule, metastasis) clinical stage and molecular classification. The result of the immunohistochemical technique is expressed as 0 (negative), 1 (+), 2 (+) and 3 (+). When our result was reporting as 0 to 1 (+), they are considered HER2 negative. If it shows a 3 (+) result it is called a positive HER2 (ERBB2 protein human), according to Wolff [6].

There were 13 cases (56.52%) with Her2/neu receptor overexpression, included in the subgroup reported as 0 and 1 (+). Most presented in TNM stage I and II (10 cases), that is, with a good prognosis. The positive cases were 10 (43.47%), divided according to the degree of immunostaining as 2 (+) and 3 (+).

They corresponded in greater number with the worst prognosis stages (III and IV), with 7 cases.

In the summary of Table n° 1, group 0/3 (negative) has the highest number of cases, with eleven. Likewise, clinical stage I, represented by five cases, is the one with the highest percentage in this subgroup. The 2/3 cases, also reported as 2 (+), are those with the highest representativeness in the positive Her2/neu subgroup, with six cases. In this subgroup, the most frequent TNM clinical stage is III.

According to the molecular classification, we distribute the patients into four subgroups (Her/neu positive, triple negative, luminal A and Luminal B). It is consistent with the expression of the Her2/neu receptor and other proteins, such as the estrogen and progesterone receptor.

In table 2 we show the indicators according to each category. The TNM clinical stage is correlated to the probability of death, depending on the overexpression or not of the HER2/NEU receptor. The decision rule is to reject the null hypothesis of correlation between positive cases and the poor prognosis clinical stage (III and IV).

The evaluation of the global survival, we made it through the Kaplan-Meier curves (Figure 1). It is described as a graph of the survival curve, in which the ratio is on the vertical axis and time on the horizontal. Each ladder corresponds to the "death" event. The upper curve of Her2/neu negative cases shows a more favorable survival experience.

Since the Kaplan-Meier curves are designed to graphically represent the survival analysis, we require additional testing to establish statistical significance. Precisely, we carry out this statistical concept with the Logrank test (Mantel-Haezel). We evaluated the decision to reject the null hypothesis, only if it indicates that there is no difference in survival between cases of patients with positive and negative Her2 / neu immunohistochemical results. We avoid making a type I error (false positive) if this null hypothesis is rejected as true. If the p-value, given by the Logrank test, is lower than the significance level, then the null hypothesis must be rejected (p <0.05). Since this value is 0.017, the null hypothesis is rejected.

3.2. Immunohistochemical Study

Immunohistochemical staining was carried out with the Antibodies Method (Dako), as previously described. All slides were examined by the researcher and another pathologist. In Figure 2 we present a case with Her2/neu receptor overexpression. The monoclonal antibody is deposited in more than 90% of the cell membrane, delimiting cell spaces, in which the nucleus is not observed. Some neoplastic cells have giant shapes with dividing nuclei, which is characteristic of most undifferentiated neoplasms.

The immunostaining pattern report is done like this:

- -0: Her2/neu negative.

-1/3: Her2/neu negative.

-2/3: positive for Her2/neu over-expression.

-3/3: positive for Her2/neu over-expression.

The sheets reported as 2 (+) have a peculiarity. They are thus grouped only when there is deposition of the antibody with complete staining on the cell membrane, covering between 10% to 30%, of the cytological component seen under the microscope. Six samples were reported in our study. Only Her2/neu 2 (+) require confirmation with FISH [7], due to the possibility of being a false positive. This technique has greater sensitivity and specificity. Recently, a new method based on the quantitative amplification of Her2 by PCR (Polymerase Chain Reaction) has been introduced, which will possibly become the standard test in the near future, due to its speed and concordance [8].

4. Discussion

The overexpression of the HER2 oncogene, reported by the researchers, indicates a decreased OS in breast cancer. The study of the oncogene in the world is evaluated by immunohistochemistry [3, 6, 10]. The economic cost of the technique makes it inaccessible to low-income patients, such as Peru's indigenous ethnic groups, partly due to the few institutions that do it

The immunohistochemistry technique in Peru is feasible to be performed, according to our research. The primary reaction between the antigen and the antibody for HER2/neu is amplified with a secondary antibody biotinylated. This chemical reaction is demonstrated with the formation of the streptavidin-peroxidase complex, as well as the subsequent addition of the diaminobenzidin chromogen. When we verify it on the slide, through the microscope, a specific staining pattern is evidente.

Breast cancer is a heterogeneous disease, with a different natural history for each subtype [5]. Some researchers report that the importance of determining human ERBB2 protein, is given by its therapeutic utility, which leads to the testing of new monoclonal antibodies, alone or in combination with chemotherapy [9]. In our research, the receptor overexpression group comprises 43.47% of cases, which could benefit from the use of monoclonal antibodies. These results are similar to those found in an Australian study, where 3 (+) overexpression was 16.90% of cases [10]. In previous studies carried out in Peru, a similar percentage was found, with 41% of the 117 cases studied, 66% associated with a histological grade III [11].

The highest percentage, with 56.53%, of cases is represented by the negative Her2/neu group (0/3 and 1/3). This result is consistent with the statistics of other studies, such as Telli M, in which women of Chinese origin represent 64%, korean women with 49% and vietnamese with 57% [12]. In Arequipa (Peru) this percentage reaches 82.00% of 280 patients evaluated [13]. Global updates advise identifying these populations, as it has an impact on therapeutic orientation [4].

In other countries, the percentage of overexpression is lower than our findings, such as in Spain, where 15-20% are positive cases [14]. In Cuba, the percentage is lower, with 198 biological samples evaluated, of which 10.60% are Her2/neu overexpressed [15]. In a study carried out in China, this same group shows similarity to our results, with 65.13% (762 cases) [16].

In our results, according to the TNM indicator, we showed that Her2 / neu positive patients correlated with the worst prognosis stages (III-IV). In Spain, when all molecular types are evaluated, including luminal and triple negative, mortality rates are lower [17]. In Colombia, Her2/neu positive cases, regardless of whether the evaluation was performed by immunohistochemistry or FISH, show a worse prognosis [18]. The same is true in France, where it is a poor prognostic factor, but it predicts the response to treatment with monoclonal antibodies [19]. In Mexico, Her2/neu positive patients (25.60% of 125 cases) represented the group with the highest percentage of metastases at follow-up [20].

In Australia, Her2/neu overexpression is also associated with a worse prognosis; However, this characteristic can be reversed with the use of monoclonal antibodies [21]. This explains the importance of performing molecular staging. In Peru, clinical stages III and IV tend to have more positive Her2/neu cases, which explains why survival was 70 months, compared to the same stages in luminal A and B, with 72 months [13].

The researchers reported that OS (overall survival), compared to the Kaplan-Meier curves, plotted a greater chance of death for positive Her2 / neu cases. These results are consistent with regional and global studies. In Chile, in biological samples from 181 patients, positives for overexpression showed greater aggressiveness and less survival [22]. In Colombia, after 46.6 months of follow-up of HER2-positive patients, it was concluded that the subgroup with stage III, under 40 years of age, high histological grade and without coexpression of hormonal receptors, had a worse prognosis [18].

In India, of 90 cases evaluated, 30% corresponded to positive Her2/neu and were those that showed the worst prognosis [3]. Even this lower survival affected the early clinical stages. In Israel, of 58 cases evaluated, 21 were positive for Her2/neu (36%), and corresponded mainly to metastatic stages [23]. In Peru, according to Medina's study, the group with the lowest survival is triple negative, with 60 months, followed by Her2/neu positives with 70 months [13]. In this same study, the group with the best prognosis was Luminal A, at 72 months, with a significant difference in the LogRank test (p=0.003).

The importance of these studies is to separate the groups where the presence of the HER2/neu receptor serves to apply the therapy with monoclonal antibodies. In Europe the prognosis is improved in metastatic stages, when targeted treatment is used [24]. In this study, overall survival increased from 7.2 months to 17.1 months, when the patients received monoclonal antibodies.

We determined that the monoclonal antibody immunohistochemistry technique can be performed in the native population of Arequipa (Peru). Likewise, there was a correlation between the overexpression of the Her2/neu receptor (ERBB2 protein human), with higher mortality. This group of women tended to be classified in clinical stage III and IV, considered to have a worse prognosis.

Therefore, the performance of the technique is validated to predict a worse prognosis, if there is an overexpression of the Her2/neu receptor. The monoclonal antibodies should be used as a molecular technique in native populations (Latin America), as part of their oncological staging.

The application of monoclonal antibody technology in cancer, both for diagnosis and treatment, should benefit all populations in the world. In Latin American countries, such as Peru, the economic cost and the lack of universalization of these techniques contribute to the worse prognosis of cancer patients. Therefore, it is the researchers themselves who must generate studies in their native populations, disseminate them and generate the budget for their implementation.

Acknowledgements

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] M., Stern, L., Fejerman, R., Das, V., Setiawan, et al. Variability in Cancer Risk and Outcomes Within US Latinos by National Origin and Genetic Ancestry. Current Epidemiology Reports, 2016, 3: 181–190. https://doi.org/10.1007/s40471-016-0083-7

[2] R., Murthy, S., Loi, A., Okines, E., Paplomata, et al. Tucatinib, Trastuzumab, and Capecitabine for HER2-Positive Metastatic Breast Cancer. N Engl J Med, 2020. 382, 597-609. https://doi.org/10.1056/NEJMoa1914609

[3] K., Goud, S., Dayakar, K., Vijayalaxmi, S., Babu, V. Reddy. Evaluation of HER-2/neu status in breast cancer specimens using immunohistochemistry (IHC) & fluorescence in-situ hybridization (FISH) assay. Indian J Med Res, 2012, 135, 312–317. Available from: http://www.ijmr.org.in/text.asp?2012/135/3/312/95605

[4] M., Akram, M., Iqbal, M., Daniyal, A,. Khan, Awareness and current knowledge of breast cancer. Biological Research. 2017. 50, 33-53. https://doi.org 10.1186/s40659-017-0140-9

[5] R. R. Gamarra Manrique (1° edition). Principio de Origen del Cáncer: Filogenia Molecular. Aleph Impresiones S.R.L. Primera edición, Dinamarca, Copenhague, 2019.

[6] A. C., Wolff, M., Hammond, K., Allison, Harvey, B,. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol. 2018. 36, 2105-2122.

https://doi.org /10.1200/JCO.2018.77.8738

[7] Z., Liu, K., Wang, D., Lin, J., Xu, et al. Impact of the updated 2018. ASCO/CAP guidelines on HER2 FISH testing in invasive breast cancer: a retrospective study of HER2 fish results of 2233 cases. Breast Cancer Res Treat. 2019, 175, 51-57. https://doi.org/10.1007/s10549-019-05148-5

[8] B., Wasserman, D., Carvajal-Hausdorf, K., Ho, W., Wong, et al. High concordance of a closed-system, RT-qPCR breast cancer assay for HER2 mRNA, compared to clinically determined immunohistochemistry, fluorescence in situ hybridization, and

quantitative immunofluorescence. Lab Invest. 2017. 97, 1521–1526. https://doi.org/10.1038/labinvest.2017.93

[9] S., Swain, J., Baselga, S., Kim, J., Ro, et al. Pertuzumab, Trastuzumab, and Docetaxel in HER2-Positive metastatic breast cancer. N Engl J Med. 2015, 372, 724-734. https://doi.org/10.1056/NEJMoa1413513

[10] M., Bilous, A., Morey, J., Armes, R., Bell., et al. Assessing HER2 amplification in breast cancer: findings from the Australian In Situ Hybridization Program. Breast Cancer Res Treat. 2012, 134, 617–624. https://doi.org/10.1007/s10549-012-2093-6

[11] R., Meléndez and A, Asencio. Factores clinicopatológicos asociados a la amplificación del gen HER2/neu en pacientes con cáncer de mama HER2/NEU (positivos). Anales de la Facultad de medicina. 2017, 78, 381-385. http://dx.doi.org/10.15381/anales.v78i4.14257

[12] M., Telli, E., Chang, A., Kurian, T., Keegan., et al. Asian ethnicity and breast cancer subtypes: a study from the California Cancer Registry. Breast Cancer Res Treat. 2011. 127, 471-8. http://dx.doi.org/10.1007/s10549-010-1173-8

[13] G. Medina Características clínicas y pronósticas de los subtipos moleculares de cáncer de mama determinados por inmunohistoquímica, Arequipa, Perú. Rev. perú. med. exp. salud public, 2017. 34, 472-477.

https://doi.org/10.17843/rpmesp.2017.343.2530

[14] A. Prieto. Cáncer de mama: tratamientos inmunológicos. Clinica. 2014, 27: 33-41. https://doi.org/10.24197/cl.27.2019.33-41

[15] V., Ramírez, G., García, C,. Lores, Y., Sánchez, C., Márquez. Histological and immunohistochemistry of invasive breast cancer in Pinar del Río province. Revista de Ciencias Médicas; 2019, 23, 71-78. Available from:

http://revcmpinar.sld.cu/index.php/publicaciones/article/view/3801

[16] X, Zeng, Z, Liang, S, Wu, W., Zhou, T. Liu. HER2 status in breast cancer of Chinese women: a study of 1170 cases using fluorescence in-situ hybridization. Chinese Journal of Pathology. 2006, 35, 584-588.

https://doi.org/10.3760/j.issn:0529-5807.2006.10.003

[17] J., Ferrando, L., Palència, M., Gotsens, V., Puig-Barrachina. et al. Trends in cancer mortality in Spain: the influence of the financial crisis. Gac Sanit. 2019. 33, 229-234. https://doi.org/10.1016/j.gaceta.2017.11.008.

[18] M., Montoya, W., Gómez, J., Sánchez, H. García. Características y supervivencia de pacientes con cáncer de seno metastásico HER2-positivo en la era post-trastuzumab. Rev colomb.cancerol. 2018, 22, 112-118.

https://doi.org/10.1016/j.rccan.2018.09.002

[19] E., Kaczmarek, C., Saint-Martin, J., Pierga, E., Brain. et al. Long-term survival in HER2-positive metastatic breast cancer treated with first-line trastuzumab: results from the french real-life curie database. Breast Cancer Res Treat. 2019, 178, 505–512. https://doi.org/10.1007/s10549-019-05423-5

[20] A., Mohar, A., Alvarado, J., Torres, P., Cabrera, et al, Factores pronósticos en pacientes con cáncer de mama y metástasis cerebral como primer sitio de recurrencia. Salud Pública Méx, 2018, 60, 141-150.

http://dx.doi.org/10.21149/9082

[21] J., Daniels, S., Lord, B., Kiely, N., Houssami. et al. Use and outcomes of targeted therapies in early and metastatic HER2-positive breast cancer in Australia: protocol detailing observations in a whole of population cohort. British Medical Journal. 2017, 7 (1): e014439. doi:10.1136/bmjopen-2016-014439

[22] X., Mimica, F., Acevedo, D., Oddo, C., Ibáñez. et al. Evaluación del valor pronóstico de la relación neutrófilos/linfocitos en cáncer de mama de subtipos agresivos. Rev Méd Chile, 2016, 144, 691-6. http://dx.doi.org/10.4067/S0034-98872016000600001

[23] J., Zidan, I., Dashkovsky, C., Stayerman, W., Basher. et al, Comparison of HER-2 overexpression in primary breast cancer and metastatic sites and its effect on biological targeting therapy of metastatic disease. Br J Cancer. 2005, 93, 552–555. https://doi.org/10.1038/sj.bjc.6602738

[24] I., Witzel, E., Laakmann, R., Weide, T., Neunhoffer, et al. Treatment and outcomes of patients in the Brain Metastases in Breast Cancer Network Registry. European Journal of Cancer. 2018, 102, 1-9. https://doi.org/10.1016/j.ejca.2018.07.004

Table 1. CHARACTERISTICS OF PATIENTS WITH PRIMARY BREAST

Indicator	Category	Molecular expression of the HER2/Neu receptor*			
		0/3	1/3	2/3	3/3
		n° Cases (%)	n° Cases (%)	n° Cases (%)	n° Cases (%)
TNM staging	**-In situ**	1 (09.10)	0	0	0
	-I	5 (45.50)	0	0	0
	-II	3 (27.30)	1 (50.00)	2 (33.00)	1 (25.00)
	-III	0	1 (50.00)	3 (50.00)	1 (25.00)
	-IV	2 (18.10)	0	1 (17.00)	2 (50.00)
	TOTAL	11 (100.00)	2 (100.00)	6 (100.00)	4 (100.00)
Classification Molecular	**-Luminal A**	2 (18.00)	1 (50.00)	0	0
	-Luminal B ** **a)Her/neu (-)**	6 (54.60)	0	5 (83.30)	1 (25.00)
	b)Her/neu (+)	0	0	1 (16.70)	3 (75.00)
	-Her/neu positive	3 (27.30)	1 (50.00)	0	0
	-Triple Negative	11 (100.00)	2 (100.00)	6 (100.00)	4 (100.00)
	TOTAL				

* Her2/neu overexpression cases (positive) are reported as 2/3 (2+) and 3/3 (3+). Negative cases are reported as 0/3 and 1/3 (1+).

** The Luminal B group is divided into two subgroups, depending on whether they are positive for Her2/neu (6 cases) or negative (6 cases).
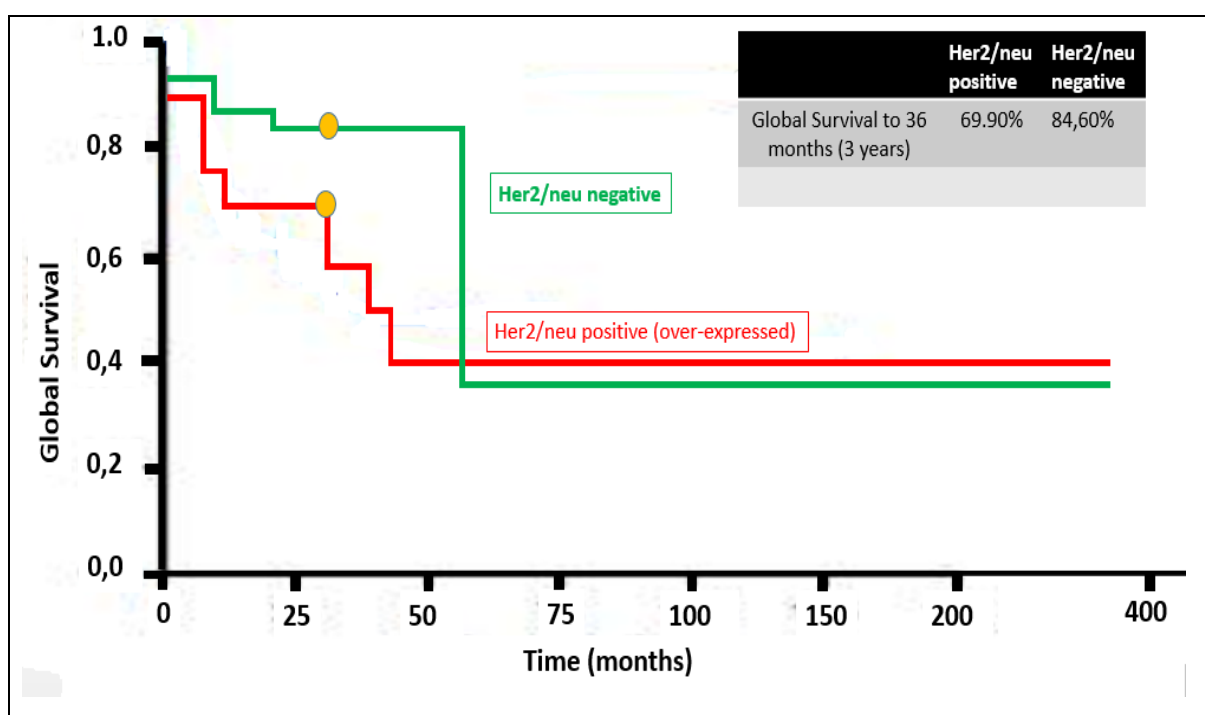
Table 2. EXACT PROBABILITY ASSOCIATED WITH THE FREQUENCY OF THE HER2/NEU

Indicator	Category	Molecular Expression of the HER2/Neu receptor		
		Positive	Negative	
				p
		n° Cases (%)	n° Cases (%)	
TNM Staging*	Early stage (in situ, I, II)	0 0 3 (30.00%)	1 (08.00%) 5 (38.00%) 4 (31.00%)	.017
	Advanced stage (III, IV)	4 (40.00%) 3 (30.00%)	1 (08.00%) 2 (15.00%)	
	TOTAL	10 (100.00%)	13 (100.00)	
Classification Molecular	-Luminal A -Luminal B -Her2/neu positive -Triple Negative	0 6 (60.00%) 4 (40.00%) 0	3 (23.00%) 6 (46.00%) 0 4 (31.00%)	.04
	TOTAL	10 (100.00%)	13 (100.00)	

* The TNM clinical stage can be divided into two subgroups: early (includes cancer in-situ, I and II) and advanced (III and IV)
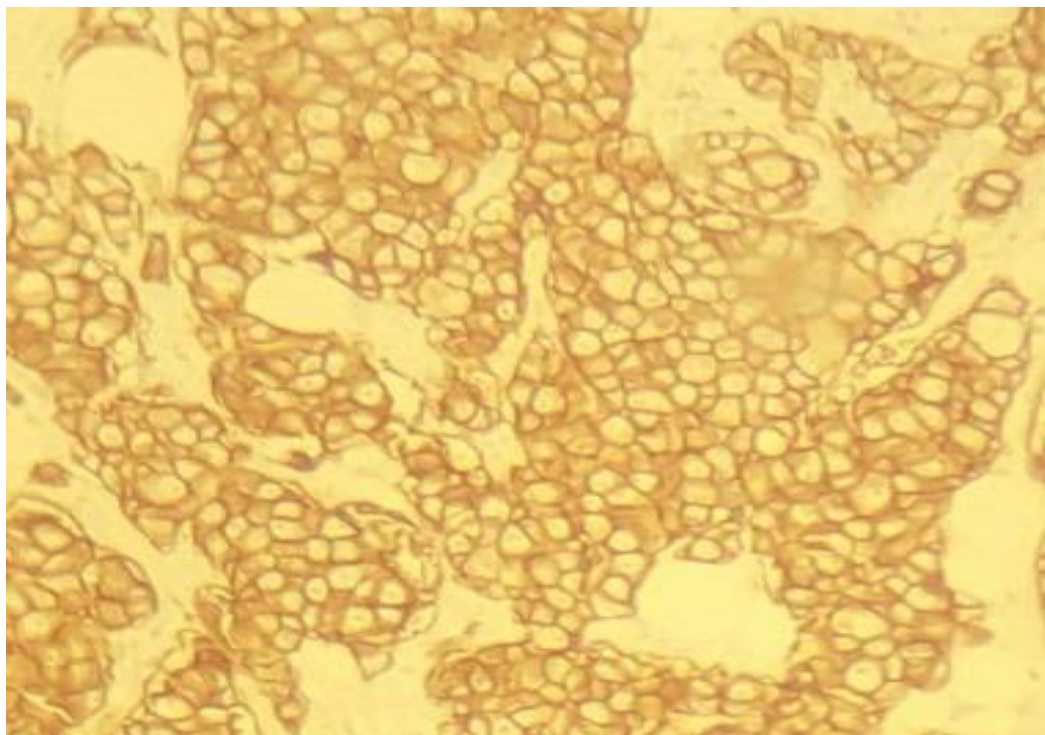
Figure N° 1

GLOBAL SURVIVAL IN BREAST CANCER HER2/NEU POSITIVE (OVER-EXPRESSED) AND NEGATIVE

The Global Survival in both groups (Her2/Neu positive and negative) is evaluated with the Kaplan-Meier curves. It derives from the Accumulated Survival ratio with respect to the result of each biological sample, together with the survival time. It is described as a graph of the survival curve, in which the proportion is on the vertical axis and the time on the horizontal.

Figure N° 2

LAMINA HER2/NEU POSITIVE IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for the HER2/neu, performed with monoclonal antibodies from mice (10×40/AN: 0,95. DAKO). The cell membrane is strongly stained in more than 90%, only in tumor cells.