

## Research Article

# HER2 Breast Cancer Group in Morocco. A Pathological and Statistical Study

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## Abstract

**Background:** The molecular classification of breast cancer is based on four parameters: HER2, Ki-67, Estrogen Receptors (ER) and Progesterone Receptors (PR).

**Methods:** We retrospectively reviewed a series of 1351 cases of infiltrating breast carcinomas in female patients diagnosed in the Pathology Department of Ibn Rochd University Hospital, Casablanca, Morocco, from 1st January 2013 to 30 March 2017. These biomarkers status was assessed by immunohistochemistry and HER2 scores 2+ were subsequently assessed by in situ hybridization. We established a molecular classification of the carcinomas and we developed a correlation profile between HER2 status and various parameters. We carried out a bivariate analysis between HER2 overexpression and SBR grade as well, in order to evaluate the degree of correlation between SBR grade and HER2 overexpression.

**Results:** Based on this study performed on a Moroccan population, we showed that HER2 molecular subgroup represents 11% of the four molecular groups and is correlated to a higher SBR grade and lymph node invasion. Close examination of the HER2 receptor reveals that it is the most overexpressed of all (35%). The average age of patients who overexpress it is 47.36 years, and women over 35 years of age are the most affected (89.86%). It is highly related to grade II (52.7%), then to grade III (44.59%), and to the presence of vascular emboli (56.75%). The bivariate study between the overexpression of HER2 and the severity of the SBR grade showed a very significant association. HER2 is correlated to SBR grade with a correlation coefficient of (Kendall tau = 0.48). It is also highly and positively correlated with the Ki67 marker (Kendall tau = 0.74). While overexpression of HER2 is negatively correlated with hormonal receptors (RE (Kendall tau = -0.16) and RP (Kendall tau = -0.04)).

**Conclusions:** As expected, we found that HER2-amplified breast tumors are characterized by an increased proliferation rates, high histologic and nuclear grades, low ER and PR levels and a strong correlation between the overexpression of HER2, and SBR grades II or III. This is, to our knowledge, the first statistical study of the kind in Morocco and North Africa.

**Keywords:** Breast Cancer; Hormonal Receptors RE and RP; HER2; Ki67; Molecular Classification; Statistical Correlation; Prognosis

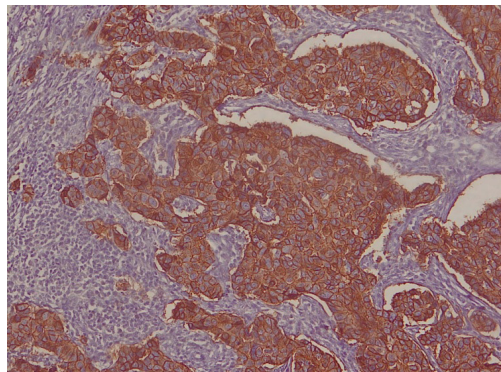
## Background

Breast cancer represents the first female cancer in terms of frequency, making it a public health problem in the four corners of the globe as well as in Morocco where breast cancer holds the first place with a standardized incidence of 30 per 100,000 women

per year [1]. It represents 36.1% of all female cancers [1]. As for the regional scale, the Cancer Registry of the Greater Casablanca estimates the incidence of breast cancer at 35.8% with a peak recorded for the 55-59 age group (2008-2012) [2].

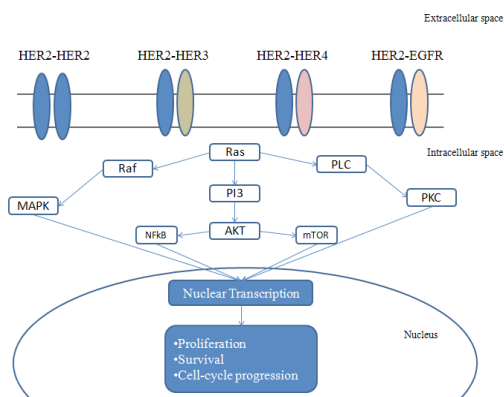
HER2 is a proto-oncogene located on chromosome 17q21 encoding for a member of the Epidermal Growth Factor (EGF) receptor family of receptor tyrosine kinases. HER2 gene can undergo various types of alterations in breast cancer, such as gene amplifications that result in the overexpression of the HER2

receptor, or point mutations resulting in constitutive activation of the receptor [3]. HER2 gene is amplified in 15% to 25% of cases of breast cancers (Figure1) [4].



**Figure 1:** Immunolabeling with anti-HER2 (Hercep Test Dako, manual technique), Magnification x 10.

HER2-amplified breast cancer is characterized by an increased proliferation rates (Figure 2), high histologic and nuclear grades, low Estrogen Receptors (ER) and Progesterone Receptors (PR) levels, increased rates of aneuploidy, relative resistance to endocrine therapy and a remarkable response to HER2-targeted therapy [5] (Figure1,2).



**Figure 2:** Transduction pathways regulated by the four HER family members.

We analyzed in this study the pathologic features of the HER2 molecular subgroup in a Moroccan population on one hand and on the other hand, the pathologic features of tumors overexpressing HER2 receptor in this same population.

## Material and Methods

We retrospectively reviewed a series of 1351 cases of infiltrating breast carcinomas in female patients diagnosed in the Pathology Department of Ibn Rochd University Hospital, Casablanca, Morocco, from 1<sup>st</sup> January 2013 to 30 March 2017. We studied conventional histopathology parameters as well as ER

and PR status, Ki-67 and HER2 status. ER, PR and Ki-67 (MIB1) status was assessed by immunohistochemistry using Dako Link 48 Autostainer using the EP1 clone for ER and the PgR636 clone for PR. HER2 status was assessed by immunohistochemistry, either manual by using the Hercep Test kit or automated by Ventana Benchmark using 4B5 clone. HER2 scores 2+ were subsequently assessed by *in situ* hybridization.

We established a molecular classification of the carcinomas based primarily on the expression of the above-mentioned markers. We developed a correlation profile between HER2 status and various parameters, such as mean age, tumor size, SBR grade, absence or presence of vascular emboli and lymph node status and we carried out a bivariate analysis between HER2 status and SBR grade in order to evaluate the degree of correlation between SBR grade and HER2 status.

## Statistical Analysis

Biostatistics treatment was developed by R for Biostatistics using the packages Hmisc, Gplots and Corplot. The tests used are Chi-square test and Kendall test. P-values <0.05 were considered as statistically significant.

## Results

Out of 1351 cases of infiltrating breast carcinomas, HER2 were overexpressed in 479 cases (35%). ER and PR were positive respectively in 1029 cases (76%) and 952 cases (70.5%). Based on the expression of the RE, RP, Ki-67 and HER2, 48% of the tumors can be classified as Luminal A, 24% as Luminal B / HER2 positive, 9% as Luminal B / HER2 negative, 11% as HER2 and 8% as triple negative tumors.

The average age of the patients is  $50.4 \pm 11.5$  years with extremes ranging from 17 to 97 years. The most affected age group is between 40 and 50 years old. Women over 35 years are much more affected (92.37%). The average tumor size (recorded in 836 patients) is  $3.64 \text{ cm} \pm 3.2 \text{ cm}$  with 64% of cases having a size greater than 2 cm (T2 and T3 stages). Nodal invasion is determined only for 320 patients of whom 66.25% are positive. The presence of vascular emboli is determined positive only in 516 patients. Tumors of SBR II grade are the most abundant (49.59%), while grade I and grade III tumors are less frequent (24.94% and 25.46% respectively).

Regarding HER2 molecular subgroup, the average age of patients is  $47.36 \pm 11.28$  years with extremes ranging from 24 to 86 years. The most affected age group is 40 to 50 years. The mean tumor size is estimated at 3.4 cm with 81.75% of cases with a size greater than 2 cm. Node invasion status is available only for 32 patients, of which 91% are positive. SBR grade II tumors were the most frequent (52.70%), followed by grade III tumors (44.59%), while grade I tumors (2.70%) were the least frequent.

The presence of vascular emboli was determined positive in 84 patients (Table 1).

Characteristics	HER2	
Total number / percentage	148	30.89%
Mean age (years)	47,36 ± 11,28	
Mean tumor size(cm)	3,43 ± 3,6	
Grade I	4	2,7%
Grade II	78	52,7%
Grade III	66	44,59%
Presence of vascular emboli	84	56,75%

Table 1: HER2 molecular subtype and Clinical-pathological characteristics.

Correlation between molecular classification and clinical-pathological data

There was no significant difference between the age averages of the patients whose tumors belonged to the different molecular classes. The tumor size does not show a significant difference depending on the molecular classes. By correlating the molecular type of the HER2 positive tumors with the histological grade, it is noted that the HER2 group is more associated with grade II and III than with grade I. Similarly, vascular emboli are present in 57% of HER2 molecular subtype (Table 1).

Bivariate study between the level of expression HER2 and the grade SBR

In order to characterize more precisely the relationship between HER2 status and the grade of cancer in our population, we carried out a bivariate analysis by studying the variables taken in couples. This study was carried out by the R for Biostatistics software, which made it possible to obtain the data corresponding to the theoretical situation and the situation observed. In the theoretical situation, based on the numbers corresponding to the status of HER2 and SBR grades, a theoretical number corresponding to the different combinations of biomarkers / grades in the case of independence (Figure 3a). In the observed situation, data collected from patient records are shown on contingency tables showing the actual combinations (Figure 3b).

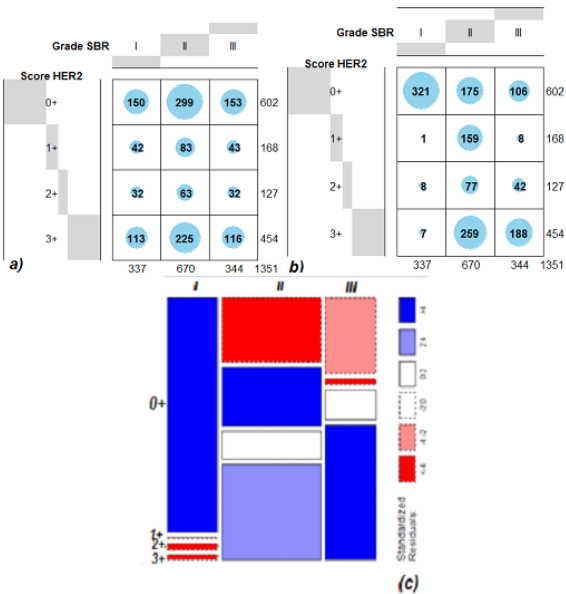


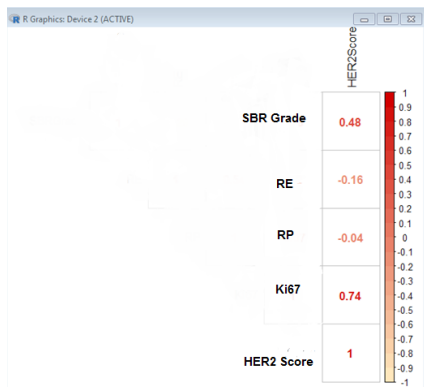
Figure 3: Graphical representation of the theoretical (a) and observed (b) population of the SBR grade as a function of the HER2 score with revelation of the difference (c) between theoretical / observed situation.

A bivariate study between the level of expression of HER2 and the grade SBR makes it possible to determine the degree of association between these two parameters. The comparison between the theoretical situation (Figure 3a) and the observed situation (Figure 3b) shows that there is a significant difference between HER2 / SBR score associations. Indeed, we note that the numbers corresponding to the cases with a HER2 0+ score associated with grade I, a HER2 score 1+, 2+ and 3+ associated with grade II, an associated HER2 3+ score to grade III, are much greater than those obtained in the theoretical situation. On the other hand, there is a decrease in the class of cases with a score of 0+ associated with grades II and III, as well as classes with HER2 scores 1+, 2+, 3+ associated with grade I (Figure 3).

Figure 3c illustrates the difference between the theoretical situation and the observed situation and confirms the results observed previously in (Figure 3b). The Chi-2 test allowed us to determine that, in our population, the association between the HER2 status and the SBR grade is extremely significant.

As shown in figure 4, HER2 overexpression is positively correlated with the SBR grade (Kendall Tau = 0.48), while it is highly and positively correlated with Ki-67 index (Kendall Tau

= 0.74). HER2 overexpression is negatively correlated with hormonal receptors (RE and RP). All correlations are highly significant (p-value < .000000), except that between HER2 and RP whose p-value is equal to 0.18, which we have considered as an artifact (Figure 4).



**Figure 4:** Correlogram between different variables.

\*Value > 0: positive correlation. Value < 0: negative correlation. The intensity of the color is proportional to the correlation coefficients. (Kendall Correlation Test)

## Discussion

HER2 is an important member of a complex signaling network and when gene amplified, it results in overexpressing HER2 protein, and therefore, results in an aggressive molecular subtype of breast cancer, the HER2 subtype. It is particularly characterized by an intense proliferation rate, while ER and PR are expressed at low levels.

In this study, we analyzed various parameters concerning 1351 patients with infiltrating breast carcinoma. The HER 2-molecular subtype represented 11% of our patients while luminal A represented 48%, luminal B 33 %, and triple negative 8%. The percentage of HER2 molecular group reported in the literature is variable ranging from 7% [6] to 20% [7], however it seems to globally account for 15% to 20% and be less prevalent than Luminal A and B subgroups [8].

The average age of patients was 47.36 years in our population. Women under the age of 35 were rarely affected, with a frequency of 10.14%. However, as reported in the literature there was no association with age for HER 2-molecular subgroup [9]. The average tumor size was 3.4 cm. 81.75% of tumors measured more than 2 cm. The tumor sizes found in our population was close to those found in other studies performed in developing countries [10,11] and were higher than those reported in developed countries [12-14]. (Table 2)

Study	Number of cases	% of HER2 molecular subgroup	Tumor size			Tumor grade			Lymph node status	
			<2	2<=5	>5cm	I	II	III	Positive	Negative
Ontilio et al. (2009) [14]	1134	7,5%	47,1%	40%	8.20%	34.10%	43.50%	22.40%	41,2%	51,8%
Blows et al. United-States (2010) [13]	10159	6%	43%	50%	7%	2%	23%	73%	58%	42%
Belhadj Algérie (2016) [11]	829	13.46%	NA	NA	NA	10%	12.10%	16.40%	12.80%	13.60%
Chahbouni Morocco (2017) [10]	390	10,0%	9%	47%	44%	9,8%	56,1%	34,1%	86.70%	13.30%
Our study	1351	11%	36%	64%		2.70%	52.70%	44.59%	91%	NA

**Table 2:** Overall distribution of Her2 molecular subtype in different populations including our study.

Most mammary tumors are thus discovered late in developing countries, whereas they are diagnosed earlier in developed countries where mammography screening is part of the national health policy. In terms of pathologic features, HER2 molecular subgroup seems to be associated with high SBR grade, lymph node and vascular invasions and thus carry a poor prognosis [10,11,13,15]. Similarly, to other studies, HER2 molecular group breast tumors in our study were moderately to poorly differentiated, they were more often grade II and III (97.29%) according to modified SBR than grade I (2.70%) (Table 2).



HER2 molecular group is frequently associated to lymph node invasion. In our population, the percentage of women with lymph node invasion represented 91%, it was higher compared to other studies, however the lymph node status couldn't be assessed for most cases because we have chosen to include also biopsies and not only surgical specimens (Table 2).

In our study we also analyzed the characteristics of breast tumors overexpressing HER2 protein. We first noticed that the rate of HER2 overexpressing tumors is high in our series (35.4%) compared to the literature (Table 3). We have hypothesized that this may partly due to the fact that HER2-positive patients of the Greater Casablanca metropolitan area are referred to our institution for treatment and HER2 status is systematically reassessed in our laboratory upon treatment initiation, with blocks of patients diagnosed outside our institution collected and reprocesses for HER2 in our laboratory. This may account in a bias resulting in higher positivity rates of HER2.

Study	Number of cases	% of HER2 overexpressing tumors
Ontilio et al. (2009) [14]	1134	17,7
Hoff et al. (2002) [21]	388	14, 2
Kim et al. (2008) [22]	338	26,3
Our study	1351	35

**Table 3:** Percentage of tumors overexpressing HER2 in different studies.

We also found that HER2 overexpression is inversely correlated with the expression of RE and RP receptors. HER2 is correlated to RE at a rate of -0.16 and with RP at a rate of -0.04 and is significant. This is consistent with the results of another study performed by Yadi, et al [16].

HER2 overexpressing tumors have been reported to be highly proliferative, and to have a high histological and nuclear grade [17,18]. This was consistent with our findings. In terms of the statistical relationship that may exist between HER2 and the SBR grade, the association was highly significant (P-value <0.00000). In other words, the higher the SBR grade is, the more likely HER2 is positive.

A correlation between HER2 receptor's expression and Ki-67 showed that it is highly significant. The correlation coefficient between these two parameters is 0.74 in our study. A significant relationship was found between Ki-67 and SBR grade, since high-grade tumors have high Ki-67 index and are therefore more invasive than other grades. These results converge with other

studies [19,20]. The Ki-67 / HER2 correlation can therefore explain the poor prognosis and the high SBR grade generally associated with HER2. A high Ki-67 index gives the tumor an important proliferative capacity resulting in a higher aggressiveness.

The strong correlation between the overexpression of HER2, and SBR grades II or III was also found in other studies: Field as and al, Paik S and al., and Jacquemier and al. They established the relationship between the expression of HER-2 and the poor prognosis of breast cancer, overall survival. In addition, the expression of this protein has been linked to resistance to hormone therapy such as Tamoxifen. These studies, as well as ours, have shown that these tumors are also associated with high rates of lymph nodal invasion, vascular emboli and the rate of distant metastasis. In contrast, hormone receptors expression is rather a good prognostic parameter since their correlation with Ki67 and the SBR grade were found to be significantly negative, therefore statistically related to a lower grade.

## Conclusion

The molecular classification of breast cancer is important for the management of patients with breast cancer, with a more personalized approach taking into account all the histological and clinical factors specific to the patient. Molecular classification has an important prognostic value as well as a predictive value for the therapeutic strategy, which allow the choice of more appropriate and more effective treatments depending on the gene or protein profile of the tumors. To our knowledge, this is the first statistical study of the kind in Morocco and North Africa. It has shed the light on some specificities of breast cancer in our country. To go further in the understanding of the dynamics of molecular breast cancer mechanisms in Morocco, it would be pertinent to suggest a prospective multicenter study in order to determine the molecular profile or even the genetic signatures of a population and to evaluate their long-term impact on the patient's prognosis, their overall survival and their response to the different therapies.

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