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HER2 STATUS BREAST CANCER IN A POPULATION OF WESTERN ALGERIA

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ABSTRACT

The HER2 gene is an oncogene encoding a human growth factor and amplified in breast cancer. Overexpression of the corresponding protein is usually the result of HER2 gene amplification secondary to oncogenic transformation. The determination of HER2 status by immunohistochemistry (IHC) allows visualization of the protein in situ: assessment and quantification of the overexpression of HER2 to target HER2 + eligible for specific therapeutic (Trastuzumab or Herceptin ®). The assessment of HER2 status is performed in correlation with clinical features, histopathological and biological mammary tumors. Material and methods: Our study from June 2007 to June 2011, 677 patients with interested invasive cancers. We used IHC method for assessing HER2 overexpression, performed on tissue blocks fixed in neutral buffered formol with the polyclonal specific antibody A 485 (Dako). Results: Age lies between 23 & 81 years old (mean age 45 ± 3 .45), histologic type is found most invasive ductal carcinoma (88%), the size pT2 (56%), grade III SBR (68%), nodal status pN + (56%), co-expression of hormone receptors ER + PR + (16%), ER + PR-(12%) RE-RP- (67%), ER-PR + (05%) . HER2 status: membrane labeling, score 0-1 + (61%), score 3 + (34%), score 2 + (05%). Conclusion:HER2 + tumors are found associated with a proportionally high SBR grade, pT 3 and ER-PR-. HER2 status does not appear to be associated with the histological type, pN or age of patients. The HER2 gene is an oncogene encoding a human growth factor and amplified in breast cancer. Overexpression of the protein is corresponding usually the result of HER2 gene amplification secondary to oncogenic transformation.

KEYWORDS: Breast cancer**,** Western Algeria**,** Status HER2**,** Immunohistochemistry.

INTRODUCTION

Breast cancer is very heterogeneous and variable in its constitution and in its fate and loco-regional treatment is influenced by this heterogeneity. Predictors of the sensitivity of a tumor to such therapy are more relevant to the choice of effective treatment adapted to the needs of the patient. Rates of estrogen receptor and / or progesterone were the first who showed the ability to respond to hormonal therapy (Bekkouche Z et al. 2000). Assessment of HER2 growth factor is an additional parameter and HER2 can be considered as a target for anti-cancer therapy (Vincent-Salmon A, 1999; Bekkouche Z et al. 2007). The gene ErbB2 (HER2) is an oncogene coding for a human growth factor and amplified in breast cancer(Prati and al., 2003). Overexpression of the corresponding protein is usually the result of HER2 gene amplification secondary to oncogenic transformation. The determination of HER2 status by immunohistochemistry (IHC) allows

visualization of the protein in situ in order to select patients eligible for HER2 targeted therapy specific anti-HER2(Rowinsky,2001;Arnould et al .,2006). The receptor 2 gene of human epidermal growth factor (HER2) is a proto-oncogene on chromosome 17q21 (Kapltain et al., 2001). HER2 is one of four growth receptors (HER1, HER2, HER3 and HER4) who have between them a high degree of homology: all these receptors have tyrosine kinase activity capable of stimulating cell growth (Fig.1) (Mounier, 2004;Hubert ,2006). The HER2 gene codes for the production of a transmembrane glycoprotein of 185 KD protein or designated as HER2 receptor consists of 5 areas (Fig. 2). The HER2 protein is normally expressed by cells seines. This receptor protein HER2 growth transmits signals from the outside to the inside of the cell and intervenes in the regulation of growth, division and cell differentiation (Fig.3) (Rowinsky,2003). HER2 protein exists in two forms in equilibrium, one is a monomer, the other is a dimer: homodimeric or heterodimeric (Fig.4). The ligand (growth factor) binds to the receptor complex in the form of dimer (Rowinsky, 2003). Overexpression of ErbB2 induces the activation and maintenance of persistent growth signals can cause malignant transformation and tumor development (Zaoui et al., 2012). This overexpression is found in breast tumors and generally corresponds to the amplification of the HER2 gene; a minority of tumors have HER2 expression without gene amplification. Antitumor activity of Herceptin ®: HER2 extracellular accessibility makes it a perfect target for antitumor treatment. In addition, the growth of tumors and human cell lines of breast cancer overexpressing HER2 is inhibited by monoclonal antibodies against this receptor as Trastuzumab or Herceptin ®. Herceptin ® should be used only in patients whose tumors overexpress HER2 (IHC) or amplify HER2 (FISH or CISH) (Couturier et al., 2000). In our study, the assessment of HER2 status is performed in correlation with the age of patients with pathological features of breast tumors and hormone receptors.

MATERIALS AND METHODS

Patients

The study was conducted from June 2007 to June 2011, concerned 677 patients with invasive cancers.

Method

To make this work, we have implemented several methods: clinical diagnosis, tumor specimen, histological and immunohistochemical study in collaboration with a multidisciplinary team.
We used immunohistochemistry (IHC) to evaluate the overexpression of the HER2 protein. The technique is performed on tissue blocks fixed in neutral buffered formalin, with the polyclonal antibody A 485 (Dako). Advantages of IHC method: This technique is widespread, it is fast, inexpensive and allows the visualization of the protein in situ (Bilous et al., 2003; Helena R. Chang, 2005).

Table 1: ***Immunochemistry technics.***

Deparaffinization blades
Blocking of antigenic sites at 90 ° C
Incubation with the primary antibody A485 (Dako)
Incubation with secondary antibody
Revelation to DAB
Counter-staining with Mayer's hematoxylin
Installation of blades

Table 2: ***Reading grid.***

Marking	Score	Overexpression
No marking or <10% of cells marked	0	Negative
Weak and incomplete marking> 10%	1+	Negative
Complete marking low or moderate> 10%	2+	Weak positive
Complete intense membrane labeling> 10%	3+	Strong positive

Reading the marking is done using a score in which the percentage of labeled cells, the staining intensity and the continuous or discontinuous marking infiltrating carcinoma cell membrane ; control internal and external indispensable (Table 1 & 2) (Penault-Llorca, 2002).

RESULTS AND DISCUSSION

Clinical characteristics: age lies between 23 & 81 years old (mean age 45 ± 3 .45). Histopathological characteristics: histologic type is found most invasive ductal carcinoma (88%), the size of pT2 (56%), grade III SBR (68%), nodal status pN + (56%). Biological characteristics (table 4): coexpression of hormone receptors (Estogen ER & Progesterone PR) ER+PR+ (15.80%), ER+PR- (11.96%) RE-RP- (67.36%), RE-RP+ (4.88%). HER2 status (table 3): membrane labelling, score 0-1 + (60.26%), score 3 + (33.97%), score 2 + (05.76%).

Table 3:*Evaluation of HER2 status by IHC*

HER2	n= 677	%
0	243	35.9
1+	165	24.36
2+	39	5.76
3+	**230**	**33.97**

High prevalence of HER2(3 +): overexpression (34%)

Table 4: *Evaluation of RE/RP status by IHC*

RESULTS HR (n= 677)			
ER+PR+	ER-PR-	ER+PR-	ER-PR+
107	456	81	3 3
15.80%	67.36%	11.96%	4.88%

High prevalence of ER-PR- (67,36%)

HER2 and tumors features

Grouping into two population HER2 (3 +) and HER2 (0) was used to facilitate our preliminary analysis, intermediate labeling were excluded.

Table 5: *HER2 and hormone receptor RH.*

HER2/HR		
HER2	ER-PR-	ER+PR+
3+ n=230	66%	24%
0 n=243	46%	48%

HER2 and RH inverse Correlation: Tumors negative for hormone receptors ER-PR- are associated with HER2 (3 +) (66%)

Table 6: *HER2/Grading SBR*

HER2/Grading **SBR**		
HER2	SBRII	SBR III
3+	27%	72%
0	65%	29%

Tumors HER2 (3 +) are found associated with a proportionally high SBR: SBR III (72%)

Table 7: *HER2/ node pN*

HER2/ node **pN**		
HER2	pN+	pN-
3+	72,7%	27,3%
0	73,5%	26,5%

The distribution of tumors according to the nodal pN is very homogeneous in the two populations HER2 (3 +) and HER2 (0)

Table 8: *HER2/ Tumour size pT*

HER2/ Tumour size **pT**			
HER2	pT1	pT2	pT3
3+	4%	59%	37%
0	16%	71%	13%

Tumors HER2 (3 +) are found associated with high size p T3 (37%) comparatively to HER2(0)(13%)

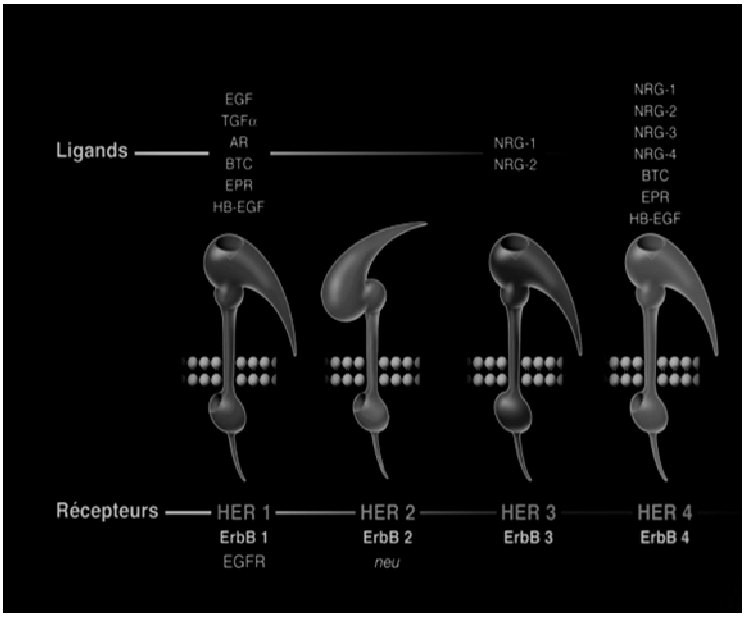

Figure 1 : ***HER Family member] (HER 1,HER2;HER 3;HER4) [Rowinsky, 2003]***

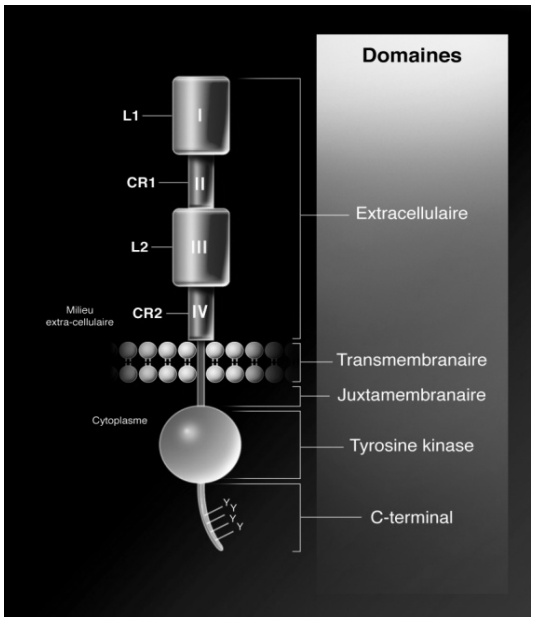

Figure 2 : ***Protein HER***

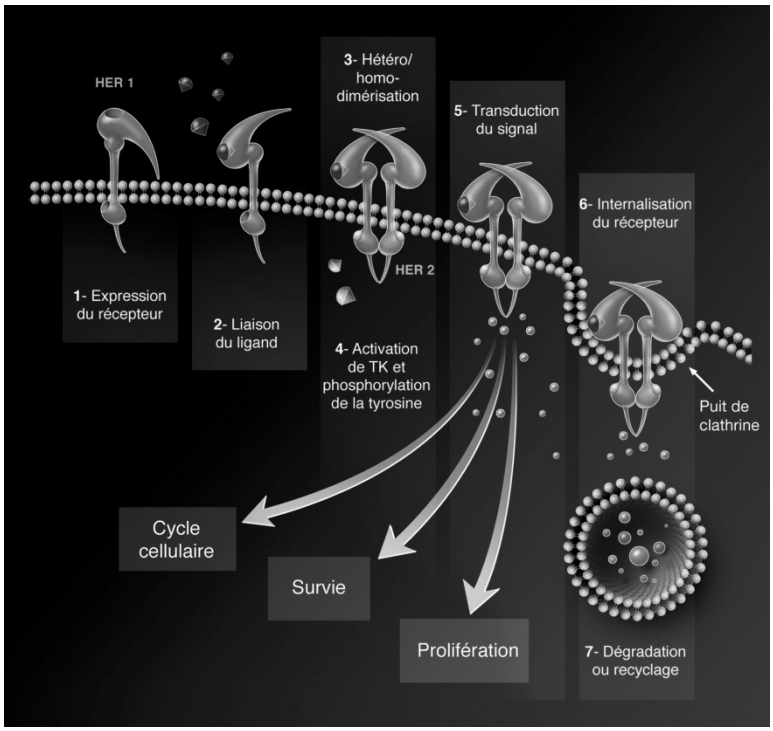

Figure 3 : HER ACTIVATION CYCLE [Rowinsky, 2003]

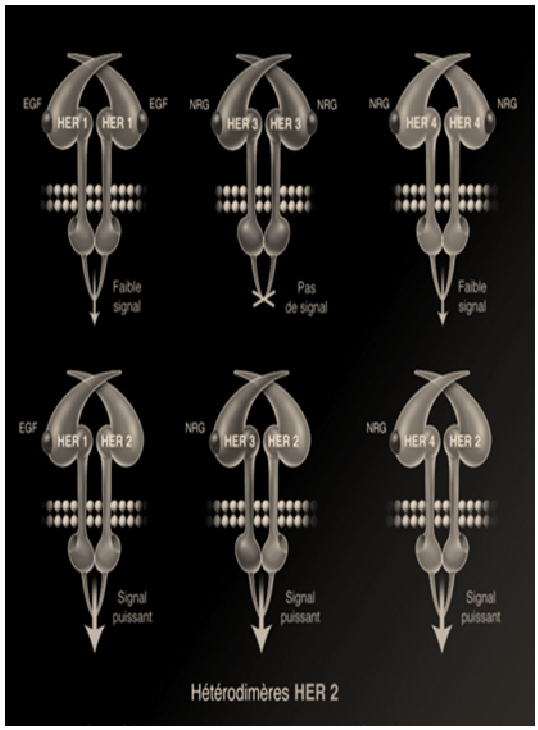

Figure 4 :Different types of signal by HER dimers [Rowinsky, 2003]

DISCUSSION

• The most common histological type was invasive ductal carcinoma (88%) associated with HER2 score 3+ and HER2 score 2+, invasive lobular carcinoma 5%
• The average age of patients was similar in the 2 populations HER2 (3 +) and HER2 (0): respectively 44.7 years and 44.4 years.
In summary, we find a positive correlation between HER2 status and tumor size, SBR grade as well as hormone receptors. In our study, the assessment of HER2 status does not appear to be associated with the histological type, nodal status and patient age.
HER2 overexpression (3 +) is a factor of tumor aggressiveness and poor prognosis in terms of survival

CONCLUSION

The HER2 status is one of the first examples of predictive and prognostic outcome study of an oncogene (Bekkouche et al., 2007). This status can be sought as routine test in the context of breast cancer as well as other tumor markers such as estrogen receptor (Bekkouche et al.,2007). Immunochemistry evaluation of HER2 has the advantage of being an easy and inexpensive, yet semi-quantitative and therefore the technique must be calibrated to give reproducible results because treatments are primarily targeted therapies in improve disease-free survival and overall survival (Vincent-Salmon et al, 2003).
It is hoped that in the future, the evaluation of alterations of oncogenes and tumor suppressor genes have a crucial place in aid diagnosis and choice of therapy in many tumors.
It is necessary before routine clinical use, standardize techniques for detecting these gene alterations and their clinical use in routine, standardized screening techniques of these gene alterations and their effect on the protein expression of these genes to a better adaptation of individual treatments (Wolff et al., 2007).

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